A Taxonomic Study of *Chrysanthemoides* Tourn. ex Medik. (Compositae)

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Abstract

A phenetic analysis based on 33 morphological characters of *Chrysanthemoides* Tourn. ex Medik. revealed 12 major clusters of operational taxonomic units (OTU's). A further four taxa were clustered within *C. monilifera* (L.) T. Norl. ssp. *pisifera* (L.) T. Norl., var. *pisifera* form 1, var. *pisifera* form 2, var. *borealis* R.C. Griffioen and var. *angustifolia* R.C. Griffioen, all eco-geographically distinct. A new coastal subspecies, *C. monilifera* ssp. *floribunda* R.C. Griffioen with two forms is erected. Characters contributing most to principal component analyses (PCA) include leaf and drupe characters, growth habit, pubescence distribution and spinescence. Results of studies on macro- and micro-morphology are discussed and illustrated.

An eco-geographical interpretation of *Chrysanthemoides* suggests that five varieties may be recognized within *C. incana* (Burm. f.) T. Norl. spp. *incana*., these being var. *incana*, var. *microfolia* R.C. Griffioen, var. *rangei* R.C. Griffioen, var. *hirsuta* R.C. Griffioen and var. *gracilis* R.C. Griffioen. The five varieties are characterised by intergrading morphologies but distinct geographical ranges. A PCA based on ecological characters, grouped three clusters in hyperspace. Distributional data separates the three clusters into 14 taxa. However, *C. monilifera* ssp. *floribunda* form 1 and *C. incana* ssp. *incana* var. *incana* are clustered together in PCA and their distribution ranges overlap. Ecological explanations for the two remaining reproductively and genetically distinct are discussed.

The results of the cladistic analysis indicate that the two species are monophyletic. *C. incana* is characterised by three non-homoplastic synapomorphies (spinescence, pungent branch terminals and prostrate growth), whereas characters of pubescence on abaxial surfaces of leaves, habit, leaf length/petiole length ratio and leaf characters support *C. monilifera*. Cladistic evidence is strong for *C. incana* being a distinct species, however, bootstrap values are low for *C. monilifera*. The cladograms grouping of 16 taxa support phenetic and eco-geographical evidence.

An isozyme electrophoretic analysis of *Chrysanthemoides* indicates that populations within reproductive and seed dispersal range are genetically similar. However *Chrysanthemoides* populations not included in this range are genetically different. High heterozygosity estimates for populations tested, may be interpreted in terms of wide seed dispersal, the large number of seedlings produced and *Chrysanthemoides* being predominantly an outcrosser.

On the basis of the phenetic, cladistic, eco-geographical and electrophoretic evidence, a new taxonomy for *Chrysanthemoides* is erected and a working key is produced to enable identification.

1.0. Introduction

1.1. The *Chrysanthemoides* Problem

Chrysanthemoides Tourn. ex Medik. is a common element of coastal shrub and fynbos, and is also found at low to mid altitudes in the mountains of southern and eastern Africa up to Kenya. It is one of the very few Compositae which produce bird-dispersed fruit that change from white through to purple-black. The showy flower and wide ecological amplitude have made the genus a popular horticultural plant for both gardens and roadsides. Some forms of the genus have been noted to be palatable, and investigations are currently under way to determine the potential of these forms as pasture plants. The importance of the genus in dune stabilization and the revegetation of mined areas has resulted in its extensive use by mining companies and soil conservation services. Two subspecies of *C. monilifera* were introduced into Australia: ssp. *monilifera* was recorded in Sydney in 1852, and ssp. *rotundata* (DC.) T. Norl. in New South Wales in 1908 and since then the latter has become one of the most noxious weeds along the coastline there, destroying natural vegetation (Weiss 1986). Investigations are presently under way to establish a biological control agent to be used in Australia.

The most recent taxonomic revision of *Chrysanthemoides* is that by Norlindh, dating back to 1943. In recent years, it has become apparent that his taxonomy does not adequately cope with the variation in the genus. This study therefore proposes a new taxonomy based on morphological, ecological and allozyme variation.

1.2. Generic Delimitation of *Chrysanthemoides*

Chrysanthemoides belongs to the tribe Calenduleae (Compositae) and is closely related to *Calendula* L., *Osteospermum* L., especially the genera *Tripteris* Less. and *Oligocarpus* Less.

especially the genera *Tripteris* Less. and *Oligocarpus* Less. (Nordenstam 1994). A cladistic analysis of the Calenduleae indicates that *Chrysanthemoides* plus *Osteospermum* is monophyletic, based on the presence of calendic acid in distinct amounts, a non-homoplastic synapomorphy. Nordenstam (1994) further separated *Chrysanthemoides* (n = 10) from *Osteospermum, Oligocarpus, Calendula* and *Tripteris* (n = 8,9) by means of chromosome number but *Gibbaria* and *Dimorphotheca* also have a basic number of ten.

The morphological distinction between *Chrysanthemoides* and *Osteospermum* sect. *Homocarpa* is not always clear, however, fruit morphology can be used to separate the two. Drupes of *Chrysanthemoides* have a fleshy exocarp and a hard endocarp at maturity. The endocarp is globose, ovoid or obovoid which is smooth or with more or less raised ridges, but without sculpturing. Sect. *Homocarpa* has wingless achenes with a closed apical cavity. The endocarp is flexible and the exocarp is a thin layer of tissue on the outer surface of the achene which hardens with maturity.

Chrysanthemoides may be distinguished from its closest relatives by yellow marginal and disc florets, with marginal florets being female and fertile while disc florets are sterile. Anthers are shortly tailed with ovate apical appendages which are sometimes purple-black (*C. incana* (Burm. f.) ssp. *incana*) or yellow (*C. monilifera* ssp. *floribunda* R.C. Griffioen (form 1)). Ovaries are oblong with apical placentation.

Chrysanthemoides may be spinescent or non-spinescent with pubescent or glabrous stems, leaves and receptacles (Norlindh 1943; Dyer 1975).

1.3. Taxonomic History

The first description and illustration of a species of

Chrysanthemoides Tourn. ex Medik. was by Breyne (Exotic. Cent. Pr.: 155, tab. 76 (1678)), who described *C. incana* (Burm. f.). The name *Chrysanthemoides* was coined in 1705 by Tournefort (Mem. Acad. Roy. Sc.: 237 (1705)) and since then a vast literature on the genus has accumulated. There seems to be confusion as to whether Medikus (Phil. Bot. 1: 159) or Fabricius (Enum. Meth. Pl.: 79) is the legitimate publisher of *Chrysanthemoides*. Fabricius' description seems to apply to the North American species, *Polymnia uvedalia*. Indications are that *Chrysanthemoides* Fabr. should becomes a synonym of *Polymnia* L. and *Chrysanthemoides* Tourn. ex Medik. should be conserved (Nordenstam pers. comm.). Pre-Linnaean literature is summarized in Table 1a.

Table 1a: Pre-Linnaean descriptions of *Chrysanthemoides* (see Table 1b for abbreviations of taxon names).

Author	Published Work	Species
Breyne	Pl. Exotic. Cent. Pr.: (1678)	*C.inc.inc.*
Rajas	Hist. Pl.: (1704)	*C.mon.mon.*
Tournefort	Mem. Acad. Roy.: (1705)	*C.inc.inc.*
Plukenet	Amalt. Bot.: (1705)	*C.mon.mon.*
Morison	Pl. Hist. Univ. Oxon. 3: (1715)	*C.inc.inc.*
Dillenius	Hort. Eltham.: (1732)	*C.mon.flo.F1*
Vaillant	Mem. Acad. Roy.: (1720)	*C.mon.mon.*
Linnaeus	Hort. Cliff.: (1737)	*C.mon.flo.F1*

Table 1b: Abbreviations used for taxon names

Abbreviation	Taxa
C.mon.mon.	*C. monilifera* ssp. *monilifera*
*C.mon.flo.*F1 & F2	*C. monilifera* ssp. *floribunda* forms 1 and 2
*C.mon.pis.*F1 & F2	*C. monilifera* ssp. *pisifera* var. *pisifera* forms 1 and 2
C.mon.pis.bor.	*C. monilifera* ssp. *pisifera* var. *borealis*
C.mon.pis.ang.	*C. monilifera* ssp. *pisifera* var. *angustifolia*
C.mon.can.	*C. monilifera* ssp. *canescens*
C.mon.sep.	*C. monilifera* ssp. *septentrionalis*
C.mon.rot.	*C. monilifera* ssp. *rotundata*
C.inc.inc.	*C. incana* ssp. *incana* var. *incana*
C.inc.mic.	*C. incana* ssp. *incana* var. *microphylla*
C.inc.hir.	*C. incana* ssp. *incana* var. *hirsuta*
C.inc.ran.	*C. incana* ssp. *incana* var. *rangei*
C.inc.gra.	*C. incana* ssp. *incana* var. *gracilis*
C.inc.sub.	*C. incana* ssp. *incana* var. *subcanescens*

The taxonomic history of the genus is summarized in Table 2. Linnaeus and Bergius recognized the same two taxa, however, Bergius (Descr. Pl. Cap.: 330 (1767)) subdivided *C. monilifera* ssp. *pisifera* (L.) T. Norl. into *Osteospermum ciliatum* and *O. pisiferum* which are synonymous with *C. monilifera* ssp. *pisifera*. Burman added a new species to the list of known species. He described the well-known *C. incana* in Prodr. Fl. Cap.: 29 (1768) based on Oldenland's much older collections. Surprisingly Thunberg, despite his extensive collection did not add any new taxa. The foundation of the modern taxonomy of *Chrysanthemoides* was established by De Candolle (Prodr. Regn. Veg. 6: 460) in 1836, who recognized nine species and five varieties, five of these species new. His taxonomy was based on the collections of Drège, and Ecklon and Zeyher who extensively collected within South Africa. Harvey and Sonder (Fl. Cap. 3: 436 (1865)) placed all the known taxa into *C. monilifera* and within this species described five varieties. No new taxa were discovered until the exploration of East Africa and Namibia. *C. monilifera* (L.) T. Norl. ssp. *septentrionalis* T. Norl., an Afromontane subspecies was described by Engler, (Abh. Akad. D. Wiss. Berlin: 447 (1892)) and *O. rangei* by Muschler (Bot. Jahrb. 56: 117 (1911)). Norlindh prepared the most recent taxonomic work on *Chrysanthemoides* describing a single new taxon, *C. monilifera* ssp. *septentrionalis* (Stud. Calend.: 357 (1943)).

Table 2: Taxa recognized in *Chrysanthemoides* (see Table 1b for abbreviations of taxon names).

Author (Date)	*C.mon. mon.*	*C.mon. flo.F1 & 2*	*C.mon. pis.F1 & 2*	*C.mon. can.*	*C.mon. sep.*	*C.mon. rot.*	*C.inc. inc.*	*C.inc. ran.*	*C.inc. sub.*
Linnaeus (1753)	*O. moniliferum*		*O. pisiferum*						
Bergius (1767)	*O. moniliferum*		*O. ciliatum* *O. pisiferum*						
Burman (1768)							*O. incanum*		
Jacquin (1798)							*O. spinosum*		
Cassini (1818)							*Eriocline obovata*		
Thunberg (1823)	*O. moniliferum*		*O. piliferum*						
De Candolle (1836)	*O. moniliferum*		*O. pisiferum* *O. ciliatum*	*O. pisiferum* var. *canescens*		*O. moniliferum* var. *rotundatum*; *O. macrocarpum*	*O. moniliferum* var. *lanosum*; *O. spinescens*		*O. subcanescens* incl. var. *virescens* & var. *angustifolia*
Drège (1843)				*O. pisiferum* var. *canescens*			*O. moniliferum* var. *lanosum*; *O. spinescens*		
Harvey & Sonder (1865)	*O. moniliferum* var. *verum*		*O. moniliferum* var. *pisiferum*			*O. moniliferum* var. *rotundatum*	*O. moniliferum* var. *lanosum*		*O. moniliferum* var. *angustifolia* *O. subcanescens*
Engler (1892)					*O. moniliferum*				
Muschler (1910)								*O. rangei*	
Compton & Pillans (1931)							*O. lanosum*		
Norlindh (1943)	*C. monilifera*	*C. monilifera*	*C. monilifera* ssp. *pisifera*	*C. monilifera* ssp. *canescens*	*C. monilifera* ssp. *septentrionalis*	*C. monilifera* ssp. *rotundata*	*C. incana*	*C. incana*	*C. monilifera* ssp. *subcanescens*

2.0. Materials and Methods

2.1. Numerical Analysis

2.1.1. Protocol

Nine hundred and twenty herbarium specimens from BOL, NBG, NU, PRE, RUH, S, STEU and LD (abbreviations follow Holmgren *et al.* 1990) were surveyed and arranged into 11 groups based on gross morphology. One hundred and thirty nine specimens were selected to represent all groups detected. Where enough material was available groups were represented by 15 specimens selected across the geographical range of each taxon. Eight *C. monilifera* ssp. *floribunda* R.C. Griffioen (form 2), ten *C. incana* ssp. *subcanescens* (DC.) T. Norl. and nine *C. monilifera* ssp. *septentrionalis* specimens were examined.

Floral morphology was assessed from mounted involucral scales and marginal and disc florets. Inflorescences were rehydrated in boiling, soapy water and mounted on a microscope slide in a 50 % aqueous glycerol solution containing 7 % fuchsin. Slides were sealed at the edges of the coverslips with nail varnish. Leaf and stem material were hand sectioned in distilled water. Cross sections of stem and leaf material were double stained for one minute using a combined Safranine-Alcian Blue stain (Tolivia & Tolivia 1987), dehydrated in a graded series of ethanol (50 %; 60 %; 70 %; 80 %; 90 %; 100 %), and finally soaked in Xylene. Sections were mounted in Canada Balsam and dried in an incubator adjusted to 70 °C. Slides were viewed after one month of drying at 40 X and 100 X magnification using a Zeiss Standard 25 and a Zeiss Axioskop. Floral morphology and stem and leaf sections were viewed and measured using a dissecting microscope fitted with a measuring graticule precise to 0.01 mm.

Leaf and floral ratios were used, rather than direct measurements, to eliminate size differences that might result from environmental modification. Each OTU was scored for 33

characters, including vegetative and floral features (Appendix 1). Character measurements were replicated three times and averaged for each specimen. Leaf and stem material was measured with a steel ruler precise to 0.5 mm, while finer characters were measured using a dissecting microscope fitted with a measuring graticule precise to 0.01 mm.

Morphological and anatomical measurement data were data-based using Foxbase+ Revision 2.00 (Christensen *et al.* 1987). Relevant data were exported via a spreadsheet into NTSYS-pc (Rohlf 1993) for principal component analysis (PCA) and cluster analysis.

2.1.1.1. Cluster Analysis

NTSYS-pc (Rohlf 1993) was used for the phenetic analyses. The data were standardized by subtracting the mean and dividing by the standard deviation so as to reduce the effect of different scales of measurement used for different characters. A dissimilarity matrix, calculated using average Manhattan distance, was subjected to agglomerative cluster as performed by the unweighted pair group method using arithmetic averages (UPGMA), a method used widely by taxonomists. The complete-link method was tested to determine whether clusters are distinct. Nested clustering patterns were displayed for the 139 OTU's in the form of a phenogram. The 'goodness of fit' of the cluster analyses were compared to the original dissimilarity matrices using the co-phenetic correlation option.

2.1.1.2. Principal Component Analysis

NTSYS-pc (Rohlf 1993) was used for the PCA. Morphological and anatomical data were standardized so as to reduce the effect of different scales of measurement used for different characters. Dissimilarity co-efficients were correlated using variables, based on standardized data. Eigenvalues and eigenvectors were

computed for the correlation matrix. Standardized variables were used to determine eigenvectors which were used as axes for the plotting of PCA. Cumulative percentages and percentages of variation accounted for were calculated for all PCA axes. The characters were projected onto one or more of these axes and a final plot of PCA produced a display of interrelationships among specimens in terms of their position in hyperspace.

Phenograms were evaluated by comparison with the material not included in the original analysis. A list and description of characters used in PCA and cluster analysis is given in Appendix 1.

2.1.1.3. The *Chrysanthemoides monilifera* ssp. *pisifera* Complex

An initial cluster analysis revealed a group of OTU's that showed weak internal clustering. Of the 36 OTU's in the group, 31 were from the *C. monilifera* ssp. *pisifera* complex and two from *C. monilifera* ssp. *monilifera*. The remaining three OTU's were intermediates of the latter and *C. monilifera* ssp. *floribunda* (form 1).

To clarify the *C. monilifera* ssp. *pisifera* complex and *C. monilifera* ssp. *monilifera*, the cluster analysis and PCA were re-run for the group. Sample size was increased to ten for each hypothesized taxon. However, only taxonomically useful characters that had eigenvalues > 0.4 in the initial analysis were used in the second analysis. Alternatively, taxonomically useful characters could have been identified using a discriminant function analysis, however, a list of eigenvalues proved this unnecessary.

2.1.2. Justification for PCA and Cluster Analysis

Many systematists have made successful use of numerical phenetics

to describe interspecific relationships in the Compositae (e.g. Jones & Young 1983; Bayer 1988, 1989; Semple *et al*. 1988; Watson & Estes 1990; Rieseberg *et al*. 1991) and other groups (e.g. Goodman 1968; Phipps 1970; Clayton 1971; Kellogg 1985; Crisp & Weston 1993). The NTSYS-pc package (Numerical Taxonomy and Multivariate Analysis System) (Rohlf 1993) may be used to demonstrate patterns of variation in a set of multivariate data. A variety of techniques are available within the package for analysing geographical variation and indicate characters that account for morphological gaps. As a result the data set is divided into a more interpretable form.

Phenetic methods are designed to reveal multiple, continuous and overlapping patterns of variation (Sneath & Sokal 1973). The problem with cladistics is the non-hierarchic nature of character state distributions, but cluster analysis can cope with this and therefore seems to be more favourable for determining infraspecific variation under a non-hierarchical model (Swofford & Belocher 1987). PCA, a non-hierarchical method, is more suitable than cladistics and cluster analysis at the population level (Crisp & Weston 1993) since no rigid hierarchical pattern is imposed on the data when none is expected. By examining the factor loadings characters which account for the maximum amount of variation can be located. Data can therefore be displayed on a two or three dimensional graph.

A PCA was used in favour of a correspondence analysis (CA) because continuous data is more efficiently handled by this method. CA on the other hand is a ordination procedure that makes used of a two-way contingency table of objects and their attributes (Hill 1973, 1974).

2.2. Eco-geographical Data

2.2.1. Protocol

2.2.1.1. Eco-geographical Data Collection

Locality, grid reference, habitat and ecological data including altitude, rainfall, vegetation and soil type were recorded for each specimen. The distribution data collected were plotted on grid reference maps for taxa (Maps 1 - 13). Rainfall, altitude, soil and vegetation type information if not available on herbarium specimens, were inferred from appropriate maps (Midgley & Pitman 1978; Weather Bureau of Southern Africa, Pretoria, 1921 - 1950), and 'Veld Types of South Africa' (Acocks 1988).

2.2.1.2. Geographical Data Analysis

As a measure of similarity between the distributions of a pair of taxa, Sørensen's quotient of similarity (QS) (Sørensen 1948) used by Exell and Wild (1961) to determine plant-pattern relationships in the Flora Zambesiaca seemed the best available. Dissimilarity in distribution between taxa may indicate deviation of one taxon from another, or both from the same 'parent' plant.

'A' represents the number of quarter degrees taxon A occurs in, and 'B' the number of quarter degrees taxon B occurs in. 'X' is the number of quarter degrees common to both taxa:

$$QS = \frac{2X}{A + B} \times 100 \quad \text{..... Equation 1}$$

Equation 1, in effect, turns the quarter degrees common to both taxa into a percentage, which may be used to compare the similarity between the distributions of taxa A and B. In cases where distributions are disjunct, QS = 0. A similarity matrix

based on the estimated data was constructed for the 12 taxa identified in the numerical section.

2.2.1.3. Ecological Data Analysis

Ecological data were standardized using NTSYS-pc (Rohlf 1993) so as to reduce the effect of different scales of measurement used for different characters. The PCA was used to establish ecological groupings of the taxa of *Chrysanthemoides*. Ecological variables used were altitude, rainfall and winter or summer rainfall, listed in Appendix 3, and inferred from the appropriate maps (Climatology Weather Bureau, Pretoria, 1921 - 1950). Altitude and rainfall were listed as numerical data while winter and summer rainfall were categorized. Each operational taxonomic unit was represented by a taxon and data was averaged for ecological variables as indicated in Appendix 3. An output of eigenvalues was produced to determine character contributions to PCA component axes. The distributions of taxa of *Chrysanthemoides* were plotted onto maps to represent the degree of overlap among taxa.

2.3. Cladistics

2.3.1. Protocol

Quantitative and qualitative morphological data were scored onto a matrix using data from herbarium specimens (see Section 2.1.1.). Qualitative characters were coded for their states (see Appendix 3 for characters and character state codings). Putative taxa separated in the numerical analysis and eco-geographical section were used as terminals. The sister group *Osteospermum* sect. *Homocarpa* (*O. ciliatum* Berg.; *O. grandidentatum* DC.) was considered an appropriate source for outgroup choice because of its morphological similarity to *Chrysanthemoides*.

The parsimony computer program Hennig86 (Farris 1988) was used to determine cladistic relationships in *Chrysanthemoides*. CLADOS Version 1.2. (Nixon 1992), a graphics package, was used to develop figures and to evaluate tree topologies. All characters were treated as additive. The data set was analysed using the routine that calculates minimal length trees using ie* or implicit enumeration. A strict consensus tree was computed using the nelsen option. A weighted tree was computed using the option 'xsteps w' where consistency indexes where rescaled as character weights. Consistency (C) and retention indices (R) were calculated for both options (Farris 1989). A bootstrap analysis was performed to test the confidence limits of nodes in the cladogram (Felsenstein 1985) using PAUP Version 3.1. (Swofford 1989).

2.4. Isozyme Electrophoresis

2.4.1. Protocol

2.4.1.1. Study Sites

The following taxa were tested electrophoretically to asses their genetic relationship. Sizes of populations were approximately 100 plants (see Fig. 11 for collecting localities):

- *C. monilifera* ssp. *floribunda* (form 1) occurring approximately 120 km west of Knysna (Still Bay - 34°55'S:22°37'E). The Still Bay population is situated adjacent to the road near the lighthouse. Plants are growing in amongst *Acacia saligna* and are densely packed on dune slopes in deep sands.

- *C. monilifera* ssp. *rotundata* found at Port Elizabeth, Skoenmakerskop (34°02'S:25°32'E). The Skoenmakerskop population is distributed alongside the road approximately 100 meters from the sea shore. Plants form dense stands in deep, dry sands.

- *C. monilifera* ssp. *floribunda* (form 1) collected at Buffels Bay (34°05'S:22°55'E) were found growing in deep sandy soils, alongside the road approximately 100 meters from the sea shore growing next to *Pelargonium praemorsum*.

- *C. monilifera* ssp. *floribunda* (form 2) was collected on the margin of a forest on the old George road (Hoekville - 34°23'S:22°37'E). Soils were sandy but humus-rich. Plants were found growing next to *Acacia saliga*.

- *C. incana* ssp. *incana* (Saldanha area - 33°05'S:18°03'E). Cutting material was taken from plants collected alongside the road. A number of the samples collected had developed from runners. All plants were growing in deep, grey sands.

Further sampling and electrophoretic testing of *Chrysanthemoides* populations in the Bredasdorp area was done. The following populations were collected:

- *C. monilifera* ssp. *pisifera* var. *angustifolia* R.C. Griffioen (Swellendam - 34° 00'S:20°25'E). Plants were collected alongside a dirt road at the northern end of Swellendam on a gentle mountain slope against the Langeberg. Plants were growing in wet Bokkeveld shales (approximate population size = 40 plants).

- *C. monilifera* ssp. *floribunda* (form 1) (Infanta - 34°28'S:20°50'E). This subspecies was found growing alongside the beach at Infanta next to the slipway in soil derived from limestone (approximate population size = 40 plants).

- *C. monilifera* putative hybrid (Malgas road - 34°13'S:20°40'E). Plants were collected in a low lying area alongside the dirt road 30 km from Swellendam to Malgas. Plants were found growing in shale derived soils. Plants had been partially grazed by livestock (approximate population size = 20 plants).

2.4.1.2. Technique

Five plants from each population were collected at the above described localities except for collections from the Bredasdorp area, where ten plants per site were collected. Cuttings of stem material were placed in Seradix B no. 1 root growth promoter and planted in soils from their growth sites. Two days later, they were treated with a liquid form of Seradix B no. 2 containing 4-(indol-3-yl)-butyric acid. Cuttings were replanted in a well aerated, nutritive growth medium and watered using a fine mist spray at 10 min. intervals inside a growth tunnel. The temperature was adjusted to 24 °C. Plants had grown sufficiently one and a half months later for cutting of experimental material. Young stem and leaf material was used for isozyme analysis.

2.4.1.3. Starch Preparation

Detailed methods described by Conkle *et al.* (1982), Rieseberg *et al.* (1991) and Hillis & Moritz (1990) were followed. Fifty-four grams of 'Starch Art' hydrolysed potato starch were mixed with 150 ml of gel buffer solution (see Appendix 2) in a 1.5 l vacuum volumetric flask. A glass rod was used to suspend the solution of starch and buffer. Three hundred millilitres of the buffer was heated to boiling point on a bunsen burner and the boiling buffer poured into a 1.5 l thick-walled vacuum flask containing the suspended solution of starch and buffer. The mixture was swirled to keep the starch in suspension and then placed on the bunsen burner stand until boiling point was reached. The flask was stoppered and vacuumed to degas the starch solution. A rapid effervescence resulted. This was stopped once the large bubbles had boiled through. The vacuum was released slowly to prevent further formation of bubbles and the starch solution poured into the moulding trays measuring 16 cm X 23 cm. Moulds were filled with one continuous pour until the starch slightly mounded above the edges of the mould. The gel was allowed to cool for 10 to 15 minutes at room temperature and refrigerated at 10 °C for one hour.

2.4.1.4. Gel Preparation and Loading

Trial runs indicated that Tris-Malate and Tris-HCl grinding buffers produced the best staining results. The minimum quantity of grinding buffer was used to saturate paper wicks since too much diluted the enzyme solution, and diminished the resolution of enzyme bands. The 25 samples were ground using a mortar and pestle with 11 drops of grinding buffer. Samples consisted of a paper wick (12 X 3.5 mm, Whatman chromatography paper, no. 3 MM) saturated with the ground tissue in grinding buffer. Wicks were stored at -10 °C before being loaded into gels. Each paper wicks was kept in its own phials so as to prevent cross contamination. Gels were loaded by vertically slicing the gel 3 cm from the edge of the mould along a straight edge held in line with the two origin marks on the edges of the mould. The smallest section of the gel was pushed toward the edge of the mould using light pressure of both hands until a gap of approximately 1 cm separated the two sections of gels. Paper wicks were removed from their phials and loaded onto the freshly cut surface of the larger section of the gel until the bottom section of the paper wick touched the glass bottom of the mould. Wicks from the same population were loaded evenly spaced out, alongside one another. Once all the wicks had been loaded, the smaller section of gel was pressed up against that of the larger section. A plastic straw was inserted between the bottom edge of the mould and the smaller section of the gel. This ensured that there was a continuous flow of current across the gel and prevented wicks from shifting from their original positions. Gels were covered with plastic wrap to prevent dehydration of the starch and the inserted wicks.

2.4.1.5. The Setting Up of the Gel and Buffer trays

Gels were run using a 600 volt, direct current power pack. The origin of the gel was positioned on the buffer tray at the cathodal electrode. The plastic wrap was folded back so that the

nylon reinforced, cellulose kitchen sponges could be placed in contact with approximately 2 cm of the gel at both electrodes. Sponges were soaked in the tray buffer several hours before running the gel. These were kept in plastic bags in a freezer for re-use for the same gel and tray buffer systems. Electrode trays were filled with tray buffer ensuring that the buffer level was higher than that of the palladium wire. Gel running times were recorded so as to give consistent migration distances on different days of running. Buffer system A was run at 75 mA, buffer system B and C at 70 mA. The ammeter reading was periodically checked to ensure that the amperage remained at recommended levels.

2.4.1.6. Gel Scoring and Measures Used

Interpretation of the band patterns comprising the zymograms required the knowledge of the subunit structure and the genetic control of the enzyme system. Interpretation of the genetic basis of the enzyme banding pattern relied heavily on observation of gels since literature on the composition of the active subunits is rather limited. Enzyme bands were identified by their migration distance in mm from the origin when the front had migrated approximately 70 mm.

The BIOSYS-1 computer program (Swofford & Selander 1989) was used to calculate Hardy-Weinberg equilibrium values, an index of heterozygote deficiency (Fixation test D; Wright 1965), measures of genetic similarity and dissimilarity between populations (genetic identity and distance; cluster analysis (Nei 1972)), and co-efficients of population differentiation (F_{IS}-statistics; Wright 1965, 1978; Nei 1977). Deviations from the Hardy-Weinberg equilibrium were tested using a Chi-square test using Levene's correction for small sample size (Levene 1949). Wright's fixation index (F) (Wright 1965) was calculated to indicate the ratio of the number of observed to expected heterozygotes: D = (Hobs/Hexp)-1. This is a measure of the degree of inbreeding. A

cluster analysis based on unbiased genetic distances (Nei 1978) was run to demonstrate similarity between forms on the basis of allele frequencies. Genetic distance values calculated, provided an appropriate measure of genetic similarity values for such small sample sizes used. Standard errors (S.E.) were calculated for mean allele frequency and observed and expected heterozygosity to give a measure of variance.

F_{IS}, a coefficient of population differentiation, was used to measure the decrease in proportion of heterozygous genotypes in *Chrysanthemoides* (degree of inbreeding):

$$F_{IS} = \frac{H_S - H_I}{H_S} \quad \text{..... Equation 1,}$$

where H_I is a measure of the heterozygosity of individuals in a population and H_S is the expected heterozygosity of individuals in an equivalent random mating subpopulation. Equation 1 is thus a measure of the reduction in heterozygosity of individuals due to non-random mating within a subpopulation. This is referred to as the inbreeding coefficient. BIOSYS-1 (Swofford & Selander 1989) computes this F-statistic, presenting the results in the notation of Nei (1977).

2.4.2. Justification

2.4.2.1. Available Techniques

Molecular data are often preferred to morphological data for phylogenetic and systematic inference (Gottlieb 1981; Crawford 1983; Palmer *et al.* 1988), based on the large number of independent molecular characters, their selective neutrality (Kimura 1982) and the low level of non-heritable molecular variation (Hillis 1987). In contrast, morphological data offer low numbers of characters for phylogenetic and systematic

inferences usually at the specific and subspecific level. The data are functionally or developmentally correlated and much morphological variability is non-heritable. Furthermore, morphological characters converge when exposed to similar selection pressures.

Allozyme electrophoresis and random amplified polymorphic DNA (RAPD) have been used to draw phylogenetic and systematic inferences (Cosner & Gottlieb 1977; Hamrick *et al.* 1979; Crawford & Bayer 1981; Crawford 1983, 1990; Heywood & Levin 1984; Lowrey & Crawford 1985; Giannasi & Crawford 1986; Bayer 1987, 1988; Brown 1990; Rieseberg *et al.* 1991; Hadrys *et al.* 1992). These remain the most generally applicable and efficient methods that may be brought to bear on infraspecific variation.

2.4.2.2. Isozyme electrophoresis

A major revolution in understanding micro- and macro-evolutionary processes has taken place since the invention of starch gel electrophoresis (Smithies 1955) and the visualization of enzymes on gel (Hunter & Markert 1957). Isozyme electrophoresis has been used by a number of systematists to establish taxonomic limits in the Compositae (Heywood & Levin 1984; Lowrey & Crawford 1985; Rieseberg & Warner 1987; Bayer 1988, 1989; Cosner & Crawford 1990; Rieseberg *et al.* 1991).

Closely related species are best studied by examining relatively fast evolving isozyme loci. Although other available techniques may prove useful, these are usually not sensitive enough to detect sufficient changes over short time scales (approximately five million years) (Hillis & Moritz 1990). Isozymes are most suited to addressing questions at the level of populations, subspecies and species where heterozygosity is detected within individuals (Brown 1990).

Isozyme electrophoresis may also be used to determine whether dissimilar morphology in forms is due to phenotypic plasticity or genetic variability. Quantification of variation patterns between individuals in a population and between populations are made. To a lesser extent, similarities between species, by finding unique loci also allows the delimitation of species. By establishing these patterns, inferences on other *Chrysanthemoides* populations can be made.

3.0. Results

3.1. Numerical Taxonomy

Multivariate techniques provide a number of analyses available for the study of joint relationships of variables in data that contain inter-correlations in ecology and plant systematics. Three of these seem to be the most used. Principle component analysis (PCA), linear discriminant function analysis and cluster analysis (James & McCulloch 1990).

Linear discriminant function analysis which finds linear combinations of variables with maximal ability to discriminate groups of objects, does have its limitations since the analysis uses continuous data that is well summarized by variance and covariance. Applying multivariate analysis to *Chrysanthemoides* deals largely with categorical data (Appendix 1) where variance and covariance are poor summary statistics and the technique is inefficient. PCA has been used in systematic studies of the Compositae (Jones & Young 1983; Watson & Estes 1990; Semple *et. al.* 1988). A limitation of the technique is that the procedure is intended mainly for continuous data and only considers linear combinations of variables (James & McCulloch 1990).

Both the cluster analyses and PCA produced similar clustering of OTU's, however, outliers do not correspond for the two analyses. The PCA always shows separation in the first dimension whereas cluster analysis is inherently unable to accommodate secondary pattern in the data, thus causing perturbation in the primary pattern. On the basis of this, cophenetic correlation values, eigenvalues and the variance accounted for by the first three component axes will be given as an assessment of the adequacy of the techniques used.

3.1.1. Cluster analysis

The phenogram (Fig. 1) has nine major clusters above a 0.75 dissimilarity phenon line and a cophenetic correlation value of 0.708 was calculated. The cophenetic correlation value could be low due to the cluster analysis failing to fully separate all the individuals although most specimens of each taxon clustered together. To provide a consistent cut off level for groups in the phenogram a phenon line was included in Fig. 1. The line was drawn at 0.75, as at this level it cuts off groups most consistent with the subspecies off Norlindh (1943).

Cluster 1 is composed exclusively of *C. incana* ssp. *incana* var. *microphylla* R.C. Griffioen, with small leaves. Cluster 2 consists of var. *incana*, but those with large leaves. *C. incana* ssp. *subcanescens* constitutes cluster 3.

C. monilifera divided up into six major groups. Clusters 4 and 5 are composed of spp. *floribunda* forms 1 and 2 respectively. Clusters 7 and 8 are composed of Afromontane taxa, ssp. *septentrionalis* and ssp. *canescens* (DC.) T. Norl. respectively, while ssp. *rotundata* grouped separately from other coastal subspecies with similar morphology in cluster 9.

Two specimens of ssp. *canescens* and seven specimens of ssp. *septentrionalis* are included in cluster 7. These two OTU's were collected from low altitudes in parapatric areas of the two subspecies distributions in the Transvaal. OTU 114 occupies an outlier position within the *C. monilifera* cluster. The specimen (*Levyns* 6089) appears to belong to *C. monilifera* ssp. *pisifera* var. *pisifera* (form 2). However, pubescence occurs on abaxial leaf surfaces, receptacles and stems, and adaxial surfaces are glabrous. Inner involucral scale shape and petiole length are different from the rest of var. *pisifera* (form 2) examined.

While cluster 6 consists primarily of the *C. monilifera* ssp.

While cluster 6 consists primarily of the *C. monilifera* ssp. *pisifera* complex, two ssp. *monilifera* OTU's are interspersed within this group (Fig. 1 & 3). Phenogram 2 (Fig. 2), the results of an analysis of cluster 6, produced four clusters. Cluster 6a consists of ssp. *pisifera* var. *angustifolia*, and cluster 6b var. *pisifera* (form 1). Both large and small leaved form of var. *pisifera* (form 2) were included in group 6c. *C. monilifera* ssp. *monilifera*, characterized by globose/subglobose drupes constitutes cluster 6d. OTU's 3 and 4 clustered with ssp. *pisifera* var. *angustifolia* but below the 0.85 phenon line. Both specimens have shorter leaves than the rest of cluster 6a. OTU 11 clustered with OTU 15, specimens which were thought to belong to var. *pisifera* (form 1). OTU's 11 and 15 have long leaves and short petioles which are characteristic of ssp. *floribunda* (form 2). The two taxa have parapatric distributions which indicate that OTU 11 and 15 could be intermediates. OTU 14 clustered with OTU 35, both specimens have pubescent stems and long leaves. OTU 21 clusters with OTU 14 and 35 at the base of the phenogram. OTU's 14, 21 and 35 could be intermediate populations of ssp. *pisifera* var. *pisifera* form 1 and form 2, occurring in parapatric areas of their distribution.

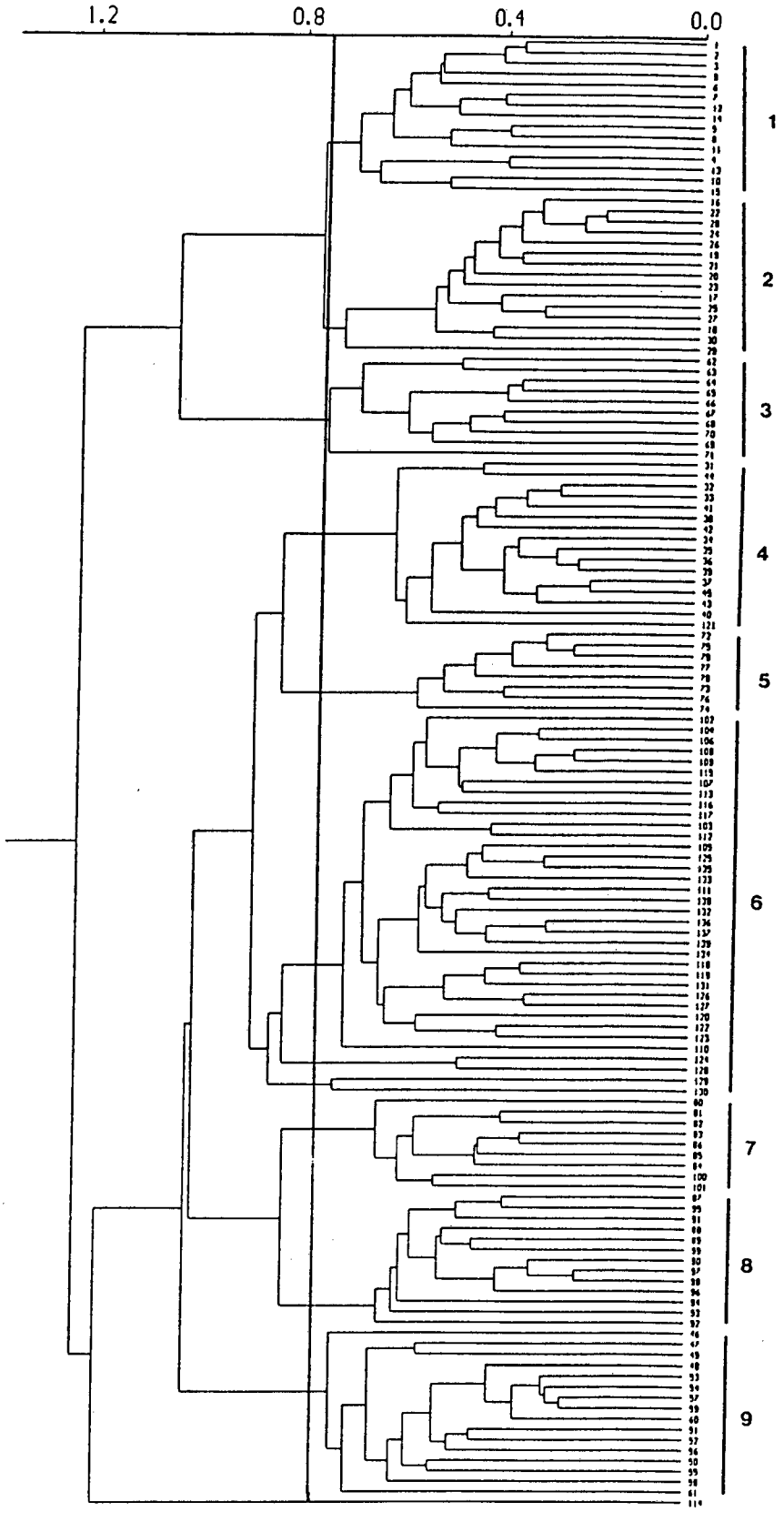


Figure 1: Phenogram for *Chrysanthemoides* showing the 0.75 phenon line. A dissimilarity axis is included. 1 - *C.inc.mic.*; 2 - *C.inc.inc.*; 3 - *C.inc.sub.*; 4 -*C.mon.flo.*F1; 5 - *C.mon.flo.*F2; 6 - *C.mon.pis.* complex; 7 - *C.mon.sep.*; 8 - *C.mon.can.*; 9 - *C.mon.rot.* (see Table 1b for abbreviations of taxon names).

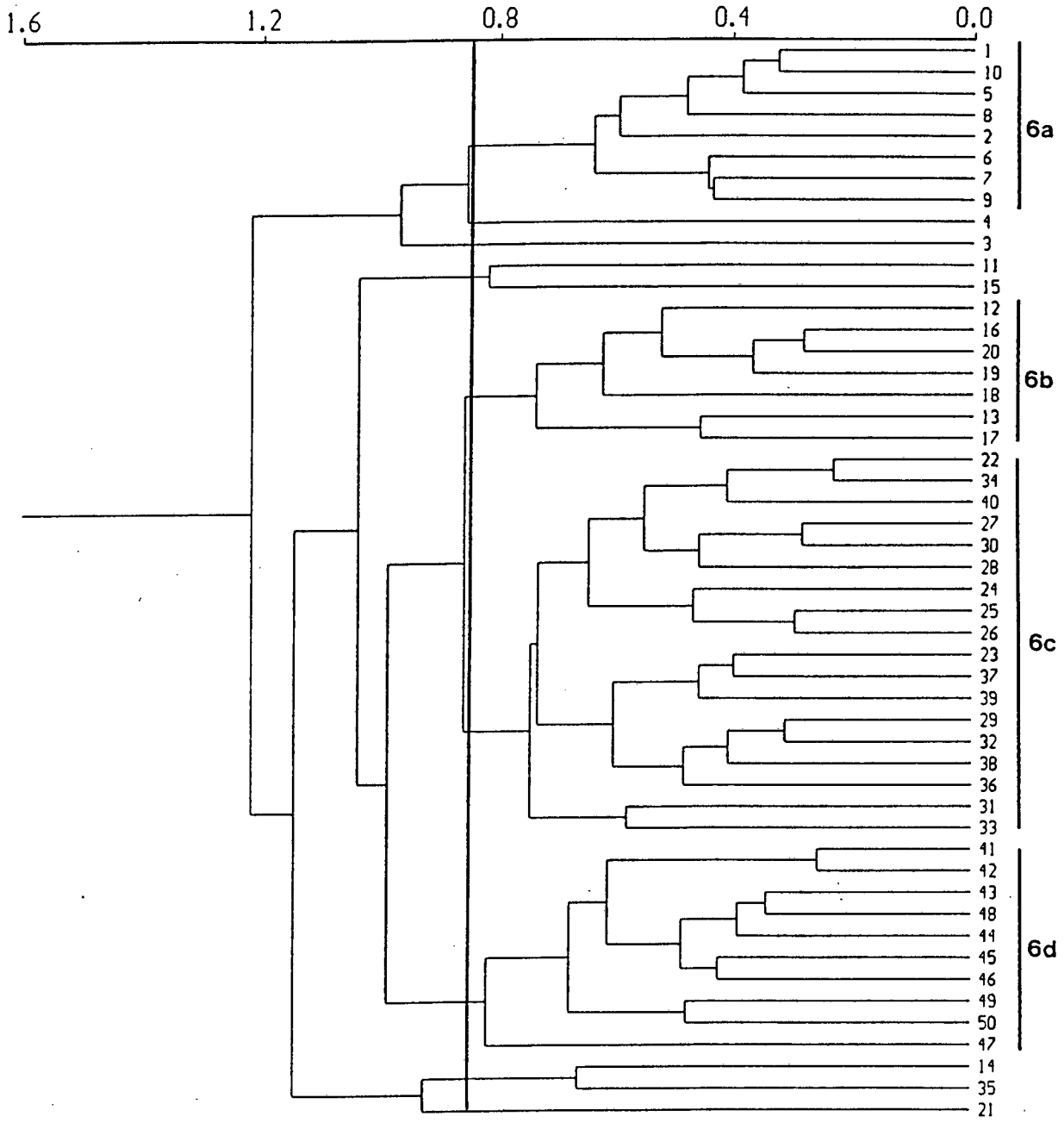


Figure 2: Phenogram for cluster 6 showing the 0.85 phenon line. A dissimilarity axis is included. 6a - *C.mon.pis.ang.*; 6b - *C.mon.pis.*F1; 6c - *C.mon.pis.*F2; 6d - *C.mon.mon.* (see Table 1b for abbreviations of taxon names).

3.1.2. Principal Component Analysis (PCA)

The first three components of PCA (Fig. 3) are fairly high and explain 44.55 % of the total variation; 21.58 %, 13.32 % and 9.65 % respectively (Table 3). These values are average, and, in a biosystematic and phenetic analysis of *Mashallia* (Watson & Estes 1990), the first three components explained 43 % of the total variation enabling the authors to separate a taxon at the subspecific level.

Eigenvalues (Table 5) for component 1 indicate that growth form (pungent branch terminals, growth habit and pubescence on abaxial and adaxial surfaces of leaves) contribute most to axis 1. High loadings for the second component are leaf margin type, leaf shape and leaf length/leaf thickness ratio and for the third component include drupe ridging, leaf length/leaf breadth ratio and drupe length/drupe breadth ratio. Eigenvalues indicate that there is variance within the data. There is overlap between the nine clusters in PCA (Fig. 3) which in general correspond to the nine clusters in the phenogram. The clusters representing *C. monilifera* ssp. *rotundata*, *C. incana* ssp. *subcanescens*, *C. monilifera* ssp. *canescens*, *C. incana* ssp. *incana* var. *microphylla* and var. *incana* are most distinct. *C. incana* ssp. *incana* var. *incana*, forms a separate cluster from var. *microphylla*. *C. monilifera* ssp. *pisifera* var. *borealis* R.C. Griffioen is incorporated into the *C. monilifera* ssp. *pisifera* complex (Group 6) which is not clear in the phenogram (Fig. 1). However, in PCA (Fig. 3) the variety clusters on the margins of the *C. monilifera* ssp. *pisifera* complex. The variety is isolated primarily by eco-geographical data. *C. monilifera* ssp. *rotundata* clusters with ssp. *floribunda* (form 1 & 2) which is not the case in the cluster analysis. Similarity in morphology and eco-geographical data suggest that PCA produces a better representation of the results than the cluster analysis.

The PCA (Fig. 4) of the *C. monilifera* ssp. *pisifera* complex and ssp. *monilifera*, indicates marginal overlap between three of the

taxa. The first three components of PCA are high and explain 51.28 % of the total variation; 23.87 %, 16.89 % and 10.52 % respectively (Table 4). High loadings (Table 6) for component 1 are characters of teeth number, leaf length/leaf breadth ratios and drupe ridging, for the second components include leaf margin type, pubescence distribution on receptacles and marginal floret length/marginal floret breadth ratio. High character loadings for the third component are drupe ratio, flower number and drupe ridging.

Therefore the PCA gives a good representation of morphological similarity within taxa, however, separating the forms and subspecies on the basis of this analysis may be queried. *C. monilifera* ssp. *pisifera* form 1 & 2 have large amounts of overlap in the PCA (Fig. 4) suggesting that little morphological differentiation has occurred with ecological divergence more apparent among these forms in *Chrysanthemoides*.

C. monilifera ssp. *pisifera* var. *borealis* is segregated primarily by eco-geographical data. Plants are morphologically similar to var. *pisifera* (form 2), however, leaf margins are spinescent and not dentate. OTU's are not clustered separately in the phenogram (Fig. 1) and PCA (Fig. 3). Most of the OTU's cluster separately with the exception for two C. monilifera ssp. *pisifera* var. *pisifera* (form 2) outliers.

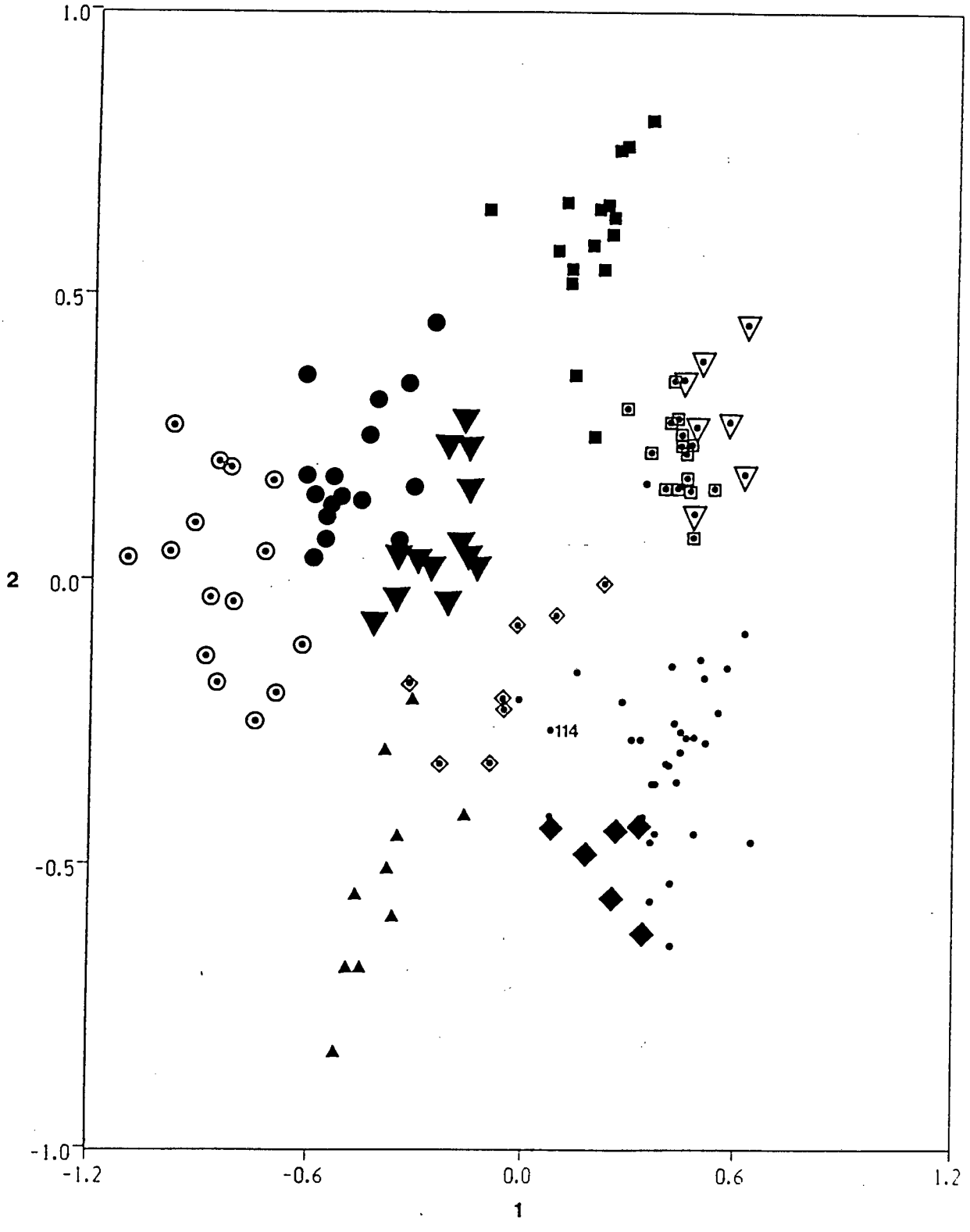


Figure 3: A principle component biplot for *Chrysanthemoides*. ⊡ - *C.mon.flo.*F1; ▽ - *C.mon.flo.*F2; • - *C.mon.pis.* complex; ♦ - *C.mon.pis.bor.*; ▼ - *C.mon.can.*; ◈ - *C.mon.sep.*; ■ - *C.mon.rot.*; ● - *C.inc.inc.*; ⊙ - *C.inc.mic.*; ▲ - *C.inc.sub.*; 114 - one of the outliers (see Table 1b for abbreviations of taxon names).

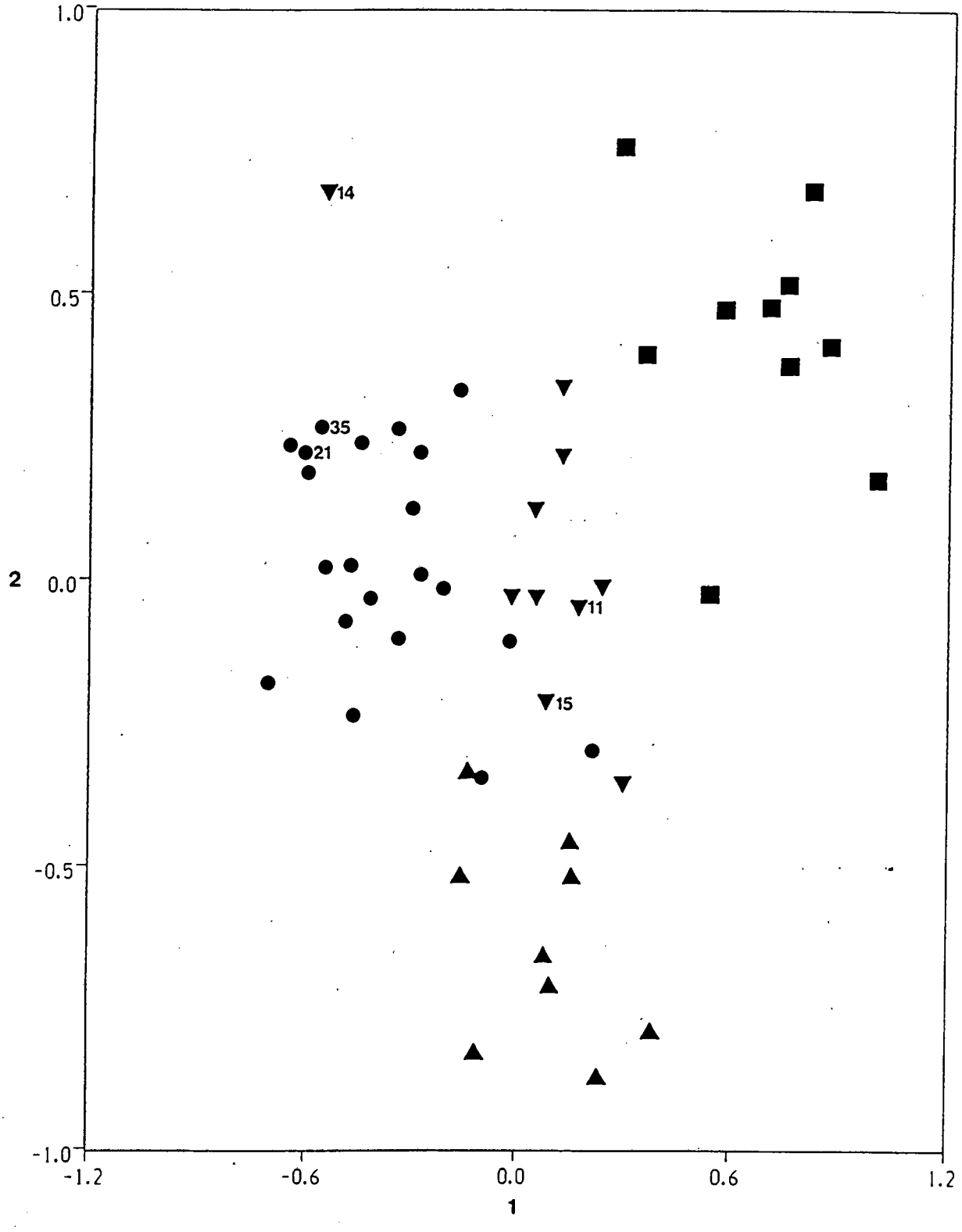


Figure 4: A principle component plot for *C. monilifera* ssp. *pisifera* and *C. monilifera* ssp. *monilifera*. ▲ - *C.mon.mon.*; ▼ - *C.mon.pis.*F1; ● - *C.mon.pis.*F2; ■ - *C.mon.pis.ang.*; 3; 4; 11; 14; 15; 21; 35 - outliers (see Table 1b for abbreviations of taxon names).

The PCA of *Chrysanthemoides* was performed on the basis of a correlation matrix. The first three factors explain 44.55 % of the variance for PCA 1 (Table 3), and 51.28 % for PCA 2 (Table 4).

Table 3: Axis values, actual and cumulative variation percentages for axes, for the whole *Chrysanthemoides* data set (Fig. 3). The third axis cumulative percentage is indicated in bold.

Axis	Axis Value	Actual Percent	Cumul. Percent
1.	7.122	21.61	21.58
2.	4.395	13.32	34.90
3.	3.183	9.65	**44.55**
4.	2.254	6.83	51.38
5.	1.687	5.11	56.49
6.	1.402	4.25	60.74
7.	1.287	3.90	64.64
8.	1.174	3.56	68.20
9.	1.151	3.49	71.68
10.	1.087	3.29	74.98
11.	0.986	2.99	77.97
12.	0.739	2.24	80.21
13.	0.730	2.21	82.42
14.	0.627	1.90	84.32
15.	0.613	1.86	86.18
16.	0.575	1.74	87.92
17.	0.539	1.64	89.55
18.	0.465	1.41	90.96
19.	0.435	1.32	92.28
20.	0.403	1.22	93.50
21.	0.384	1.16	94.66
22.	0.319	0.97	95.63
23.	0.289	0.88	96.50
24.	0.262	0.80	97.30
25.	0.253	0.77	98.07
26.	0.184	0.56	98.63
27.	0.161	0.49	99.12
28.	0.131	0.40	99.52
29.	0.111	0.34	99.85
30.	0.062	0.19	> 100.00
31.	0.021	0.06	> 100.00
32.	0.000	0.00	> 100.00
33.	-0.035	-0.11	100.00

Table 4: Axis values, actual and cumulative variation percentages for axes of *C. monilifera* ssp. *pisifera* complex and *C. monilifera* ssp. *monilifera* (Fig. 4). The third axis cumulative percentage is indicated in bold.

Axis	Axis Value	Actual Percent	Cumul. Percent
1.	3.103	23.87	23.87
2.	2.196	16.89	40.76
3.	1.368	10.52	**51.28**
4.	1.277	9.83	61.11
5.	1.110	8.54	69.65
6.	1.019	7.84	77.49
7.	0.797	6.13	83.62
8.	0.706	5.43	89.05
9.	0.542	4.17	93.22
10.	0.330	2.54	95.76
11.	0.268	2.06	97.82
12.	0.212	1.63	99.45
13.	0.072	0.55	100.00

Table 5: Eigenvalues for characters used in PCA (Fig. 3), for the whole *Chrysanthemoides* data set. Detailed descriptions of character measurements and descriptions of abbreviations are given in Appendix 1. The three characters with the highest loadings are indicated in bold.

Character	Axis 1	Axis 2	Axis 3
1. Outdent/indent	-0.30	-0.31	-0.54
2. Petiole length	-0.69	0.48	0.00
3. Inflorescence number	-0.52	0.36	0.18
4. Marginal floret number	-0.57	-0.06	-0.27
5. Drupe l/b ratio	0.23	-0.08	**-0.57**
6. Pubescence (ada leaf)	**0.79**	0.41	-0.21
7. Pubescence (aba leaf)	**0.79**	0.42	-0.16
8. Pubescence stem	0.72	0.36	-0.33
9. Pubescence receptacle	0.66	0.18	-0.25
10. Leaf shape	-0.14	**0.70**	-0.19
11. Drupe ridging	0.38	0.25	**-0.75**
12. Invol outer shape	0.14	0.03	0.21
13. Invol inner shape	-0.15	-0.46	-0.27
14. Leaf l/b	0.15	-0.52	**0.62**
15. Leaf l/petiole l	0.50	-0.31	0.26
16. Hairiness	-0.07	0.25	0.28
17. Marginal floret l/b	-0.41	-0.40	-0.40
18. Inn invol l/outer l	-0.09	-0.23	0.02
19. Marginal floret l/cap l	-0.31	-0.16	-0.22
20. Marginal floret l/ovary l	-0.69	-0.24	-0.11
21. D s l/d s f l	0.12	-0.42	-0.10
22. Leaf colour	-0.10	-0.21	0.04
23. Leaf margin	-0.06	**-0.74**	-0.44
24. Spines present/absent	0.67	-0.09	0.39
25. Stem dia/pith dia	0.09	-0.38	-0.17
26. Stem dia/xylem dia	0.02	-0.50	-0.15
27. Leaf b/cuticle b	-0.01	0.04	0.05
28. Leaf l/leaf thickness	-0.27	**0.56**	0.06
29. Tannin leaf	-0.50	0.43	-0.13
30. Tannin stem	-0.59	0.36	-0.13
31. Teeth number	-0.44	0.39	0.12
32. Pungent branch terminals	**-0.82**	0.05	0.42
33. Growth habit	**-0.82**	-0.05	0.42

Table 6: Eigenvalues for characters used in PCA (Fig. 4), for the *C. monilifera* ssp. *pisifera* complex and *C. monilifera* ssp. *monilifera*. Detailed descriptions of character measurements and descriptions of character abbreviations are given in Appendix 1. The three characters with the highest loadings are indicated in bold.

Character	Axis 1	Axis 2	Axis 3
1. Flower number	0.49	0.33	**-0.48**
2. Pubescence (stem)	-0.26	0.38	0.37
3. Pubescence (receptacle)	-0.11	**0.66**	0.26
4. Leaf margin	-0.24	**-0.80**	0.03
5. Drupe ridging	**-0.69**	0.37	**-0.41**
6. Petiole length	-0.17	-0.19	0.27
7. Marginal floret l/b	0.47	**-0.54**	0.06
8. Teeth number	**0.80**	0.15	0.25
9. Leaf shape	-0.67	-0.44	0.02
10. Outdent/indent	-0.49	-0.19	0.12
11. Drupe ratio	-0.06	0.13	**-0.69**
12. Leaf l/b ratio	**0.75**	0.27	-0.23
13. Leaf l/petiole l	0.32	-0.24	0.29

3.2. Ecology and Distribution

3.2.1. Distribution of the Genus (Maps 1 - 13).

At the southern end of *Chrysanthemoides* distribution range, *C. monilifera* is distributed from the coastal belt (found growing on coastal sand dunes) up into the mountains of the Cape Province (Hottentots-Holland, Kamiesberg, Langeberg) and the Natal and Transvaal Drakensberg. *Chrysanthemoides* occurs at the edges of forests in the George and Knysna area, were the leaf lamina is thin and marginal florets are large, to the Karoo (Middelburg, Laingsburg, Witteberg) where leaves are leathery and have deeply serrated leaf margins (ssp. *pisifera* var. *pisifera* (form 2)).

The genus occurs along the Natal coastline into Mozambique and on Inhaca Island. Further north, *Chrysanthemoides* may also be found growing on mountain slopes in the Eastern Highlands of Zimbabwe, Tanzania and southern-most Kenya (Chyulu Hills, Kenya,

Zimbabwe, Tanzania and southern-most Kenya (Chyulu Hills, Kenya, *Bally* 1170 (KAJ)). *Chrysanthemoides* occurs along the coastline from Spencer Bay in Namibia to the Gouritz River Mouth. The genus also occurs from the Western Cape (Clanwilliam district) in the dry interior to Port Elizabeth (Swartkops River).

Weiss (1986) states that *Chrysanthemoides* has been introduced to New Zealand, Sicily, Australia, Tasmania and St Helena (Norlindh 1943). The plant has also become naturalised in France (Tutin *et al*. 1976).

3.2.2. Ecology

The vegetation and soil types the various taxa are associated with are given in Tables 7 and 8. Altitude and rainfall ranges into which taxa of *Chrysanthemoides* fall are given in Table 9.

Table 7: Vegetation and habitat types taxa of *Chrysanthemoides* occur in (from Acocks 1988). See Table 1b for abbreviations of taxon names.

	Taxa	Veld Type
1.	*C.mon.rot.*	1,2,3,4,5
2.	*C.mon.flo.*F1	3,6,7,8
3.	*C.inc.inc.*	3,9,10,11,12
4.	*C.inc.hir.*	12,13
5.	*C.inc.mic.*	9,10
6.	*C.inc.ran.*	9
7.	*C.inc.gra.*	10,14
8.	*C.mon.mon.*	11,12
9.	*C.mon.pis.ang.*	12
10.	*C.inc.sub.*	1
11.	*C.mon.flo.*F2	15
12.	*C.mon.pis.*F2	7,12,16
13.	*C.mon.pis.bor.*	17,22
14.	*C.mon.can.*	18,19,21
15.	*C.mon.sep.*	19
16.	*C.mon.pis.*F1	16

1. Well drained gravel in stream bed (sandstone and shale)
2. Grassland
3. Coastal dune scrub
4. Secondary areas along roadside
5. Coastal forest (Acocks veld type 1d & 2)
6. Edge of vleis
7. Fynbos on sand
8. Limestone outcrops in sandy soils
9. Namaqualand broken veld (Acocks veld type 33)
10. Strandveld (Acocks veld type 34)
11. Coastal Renosterveld (Acocks veld type 46)
12. Coastal fynbos (Acocks veld type 47)
13. Broadleaf shrub lands on coastal calcareous sands
14. Succulent Karoo
15. Edge of forests (Knysna forest (Acocks veld type 4)
16. False fynbos (Acocks veld type 70)
17. Rich soil in river gulleys
18. Mountain Renosterveld (Acocks veld type 43)
19. Drakensberg grasslands
20. Eastern Highlands grassland
21. *Protea* veld
22. Mountain Renosterbosveld

Table 8: Soil types in which the taxa of *Chrysanthemoides* occur (see Table 1b for abbreviations of taxon names).

Taxa	Soil Type
C.mon.rot.	littoral or near littoral soils; weakly developed soils; lime present or absent
***C.mon.flo.*F1**	littoral or near littoral; undifferentiated in areas without lithosols
C.inc.inc.	sandy soil grey in colour; well drained; sand over lime; granite hills or decomposing granite
C.inc.hir.	sand or limestone, however, limestone occurs further inland
C.inc.mic.	clayey soils of dolerite origin; littoral dunes, soils derived from limestone and granite
C.inc.ran.	littoral dunes, littoral or near littoral sands or sandy flats
C.inc.gra.	sandy soils of coastal plains and heavier, stony soils of the foothills
C.inc.sub.	rocky and hilly country with pockets of soil which concentrates water; sandy, gravels of river beds
C.mon.pis.ang.	sandy acid flats, clayey soil or soils derived from granite; deep soils with old vegetation (Bokkeveld shales) or limestone ridges;
C.mon.mon.	shale ridges sandy, loamy soils over limestone; TMS gravel and well drained sandy, stony loam
***C.mon.flo.*F2**	moist acid soils, a large percentage of it being organic matter
***C.mon.pis.*F1**	soils shallow, but fertile
C.mon.bor.	soils derived from quartzite, dwyka tillite or TMS; sandy or clayey shales
C.mon.can.	well drained, poor soil, granite ground, black soil, sandy loamy soil or sandstone derived soils.
C.mon.sep.	dolerite with a shallow topsoil (300 - 400 mm) and an erodible subsoil (Acocks 1988)
***C.mon.pis.*F2**	sandstone derived soils; clayey rocky soils

Table 9: Rainfall and altitude ranges in which taxa of *Chrysanthemoides* occur (see Table 1b for the abbreviations of taxon names).

Taxa	Altitude (metres)	Rainfall (millimetres)
C.mon.rot.	0 - 150	250 - 500
*C.mon.flo.*F1	0 - 50	200 - 500
C.inc.inc.	0 - 100	200 - 500
C.inc.hir.	0 - 20	300 - 500
C.inc.mic.	0 - 1000	150 - 200
C.inc.ran.	0 - 500	0 - 50
C.inc.gra.	0 - 500	50 - 175
C.mon.mon.	20 - 1000	200 - 600
C.mon.pis.ang.	200 - 1000	300 - 500
C.inc.sub.	50 - 2000	25 - 300
*C.mon.flo.*F2	0 - 500	400 - 600
*C.mon.pis.*F2	100 - 1500	25 - 300
C.mon.pis.bor.	1000 - 1500	150 - 200
C.mon.can.	1300 - 3000	350 - 600
C.mon.sep.	1500 - 2400	350 - 600
*C.mon.pis.*F1	20 - 1500	200 - 400

3.2.3. Ecological Data Analysis Using PCA

The eigenvalues for the analysis of the ecological variables are given in Table 10. The first axis is strongly correlated with altitudinal variables (highest altitude; lowest altitude; average altitude), the second with rainfall totals (highest rainfall; lowest rainfall; average rainfall), while the third with a combination of winter/summer rainfall and altitudinal variables (winter/summer rainfall; lowest altitude; average altitude).

Table 10: Eigenvalues for ecological characters used in PCA. The three characters with the highest loadings are indicated in bold.

Character	Axis 1	Axis 2	Axis 3
1. alt high	**0.84291**	0.45308	0.07916
2. alt low	**0.78669**	0.53223	**0.22877**
3. alt avg	**0.83373**	0.52045	**0.18070**
4. rain high	0.75261	**-0.56957**	-0.06562
5. rain low	0.69822	**-0.65387**	0.05779
6. rain avg	0.75886	**-0.65006**	0.00505
7. wint/sum	0.47718	0.30175	**-0.82179**

The PCA groups the items into three clusters. The first includes the high altitude subspecies, occurring in high summer rainfall areas. Both subspecies are Afromontane occurring closer to the tropics than other taxa of *Chrysanthemoides* (7 - *C.mon.can.*; 8 - *C.mon.sep.*). The second cluster includes xeromorphic subspecies and varieties (4 - *C.mon.pis.*F1 & F2; 5 - *C.mon.pis.bor.*; 11 - *C.inc.mic.*; 13 - *C.inc.ran.*; 14 - *C.inc.gra.*; 15 - *C.inc.sub.*), occurring at low to intermediate altitudes, with predominantly winter rainfall (except *C. monilifera* ssp. *pisifera* var. *borealis* (5) and *C. incana* ssp. *incana* var. *rangei* R.C. Griffioen (13) with predominantly summer rainfall). The third cluster consists of taxa occurring at low to intermediate altitudes with intermediate winter rainfall averages (1 - *C.mon.mon.*; 2 -

*C.mon.flo.*F1; 3 - *C.mon.flo.*F2; 6 - *C.mon.pis.ang.*; 9 - *C.mon.rot.*; 10 - *C.inc.inc.*; 12 - *C.inc.hir.*). See Table 1b for abbreviations of taxon names.

The distribution ranges for members of each cluster (Figs. 6 - 8) indicate that within each cluster the elements are largely parapatric. In cases where distributions are sympatric, taxa may be ecologically separated by morphological and ecological characters not used in the analysis.

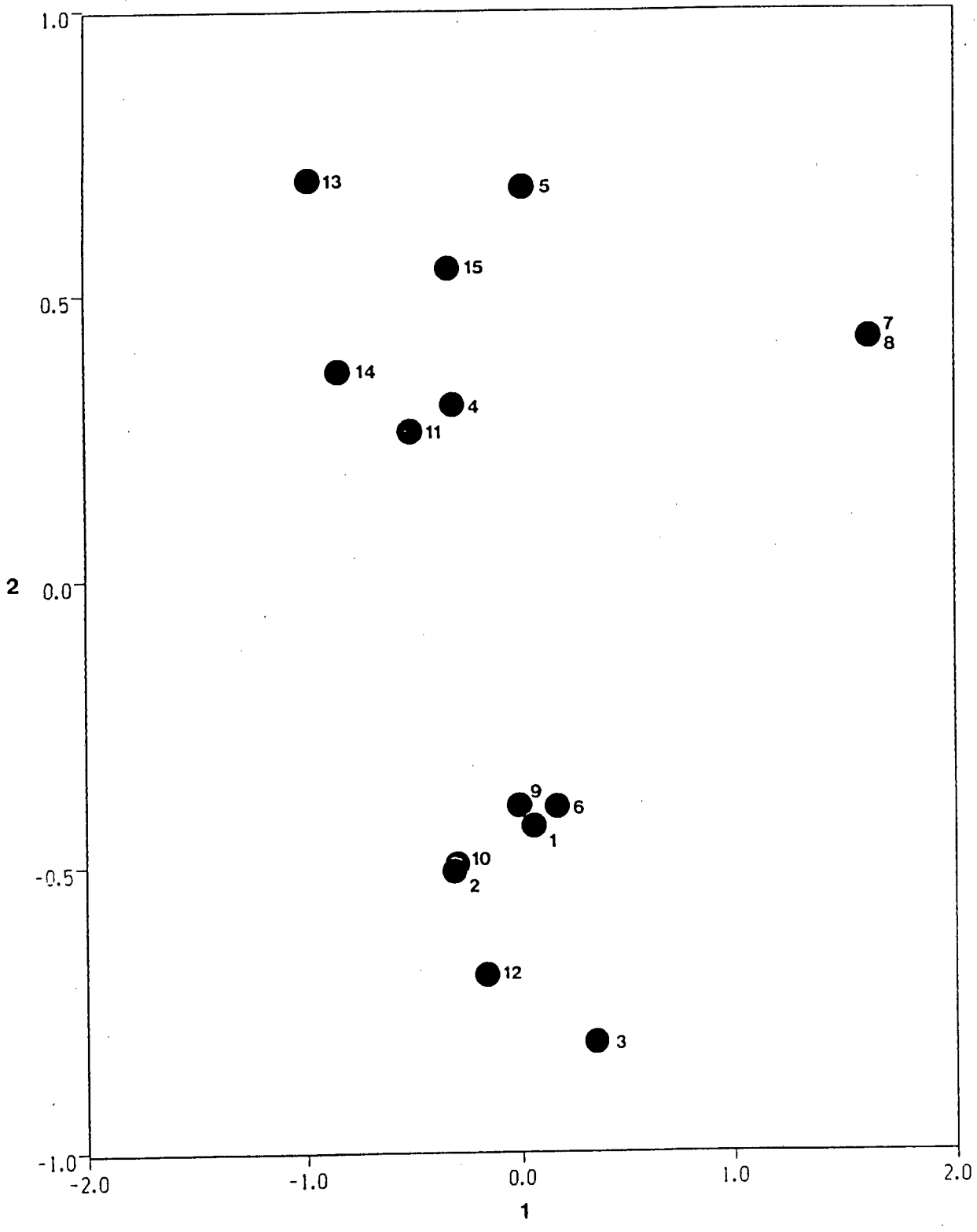


Figure 5: PCA of ecological data. 1 - *C.mon.mon.*; 2 - *C.mon.flo.*F1; 3 - *C.mon.flo.*F2; 4 - *C.mon.pis.*F1 and F2; 5 - *C.mon.pis.bor.*; 6 - *C.mon.pis.ang.*; 7 - *C.mon.can.*; 8 - *C.mon.sep.*; 9 - *C.mon.rot.*; 10 - *C.inc.inc.*; 11 - *C.inc.mic.*; 12 - *C.inc.hir.*; 13 - *C.inc.ran.*; 14 - *C.inc.gra.*; 15 - *C.inc.sub.* (see Table 1b for abbreviations of taxon names).

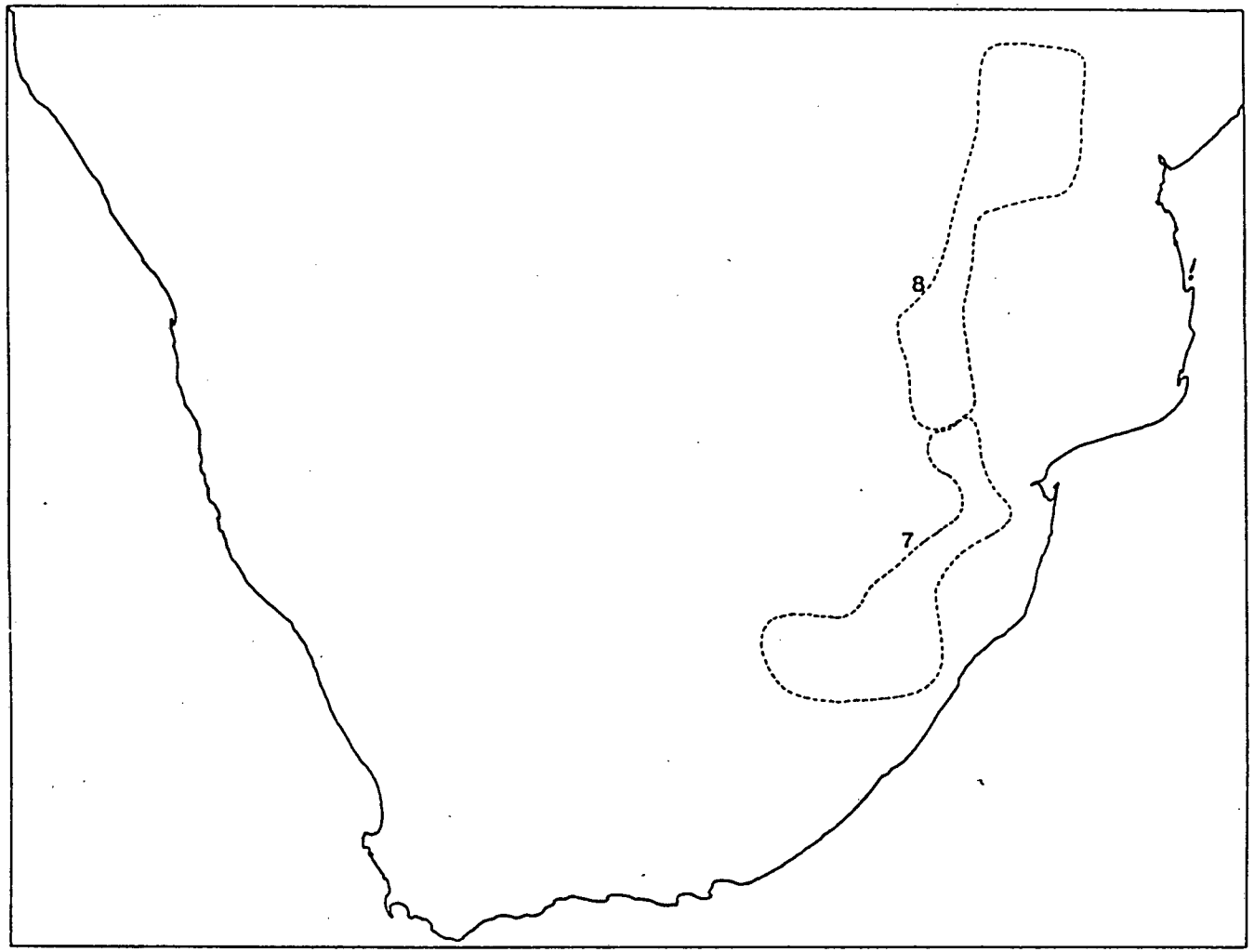


Figure 6: Distribution range outlines for 7 - *C.mon.can.* and 8 - *C.mon.sep.* The figure indicates that the two subspecies are largely allopatric but parapatric in the Pilgrims Rest area (see Table 1b for abbreviations of taxon names).

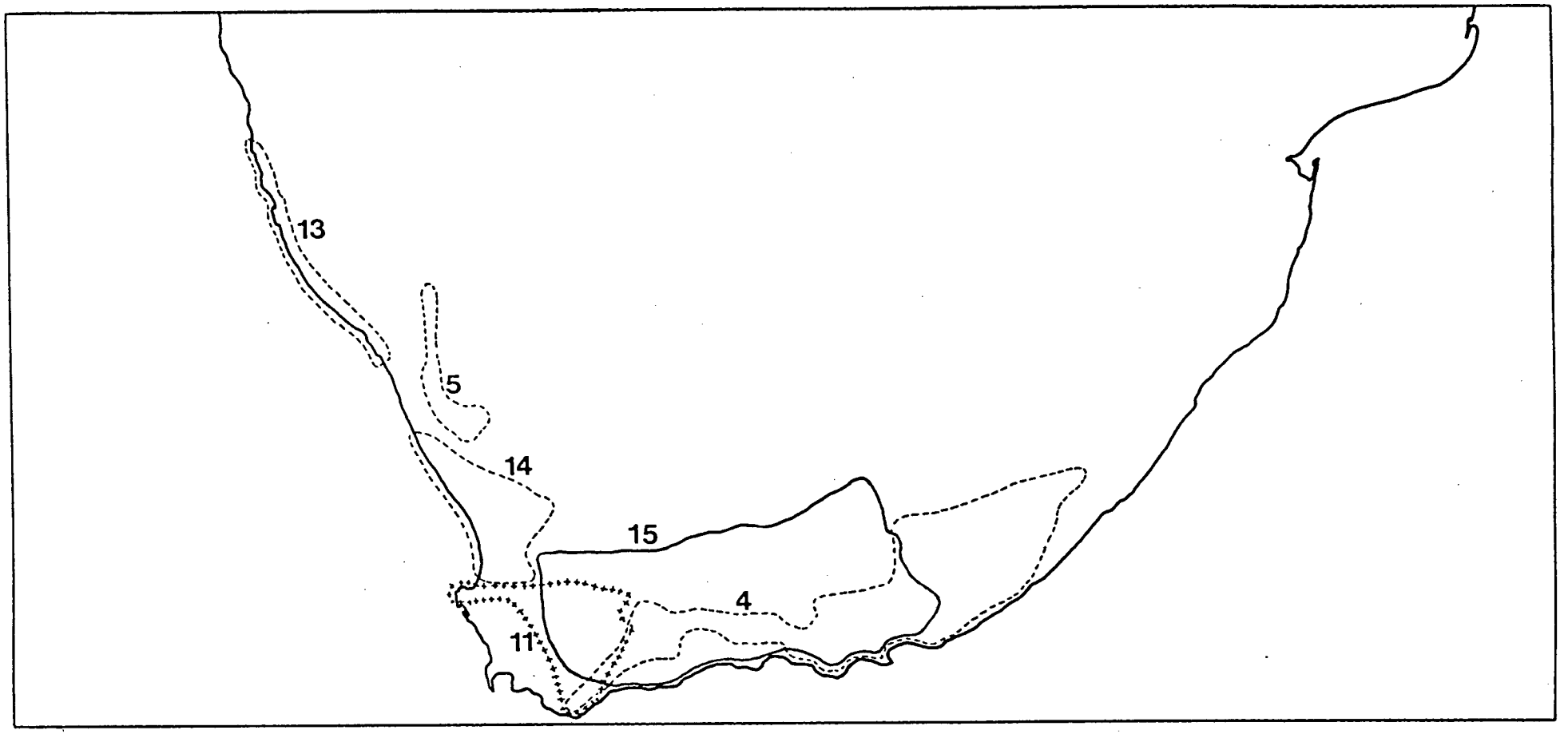


Figure 7: Distribution range outlines for 4 - *C.mon.pis.*F1 and F2, 5 - *C.mon.pis.bor.*; 11 - *C.inc.mic.*; 13 - *C.inc.ran.*; 14 - *C.inc.gra.*; 15 - *C.inc.sub.* Taxa occur in coastal vegetation where dotted lines cross terrestrial-aquatic boundaries (see Table 1b for abbreviations of taxon names).

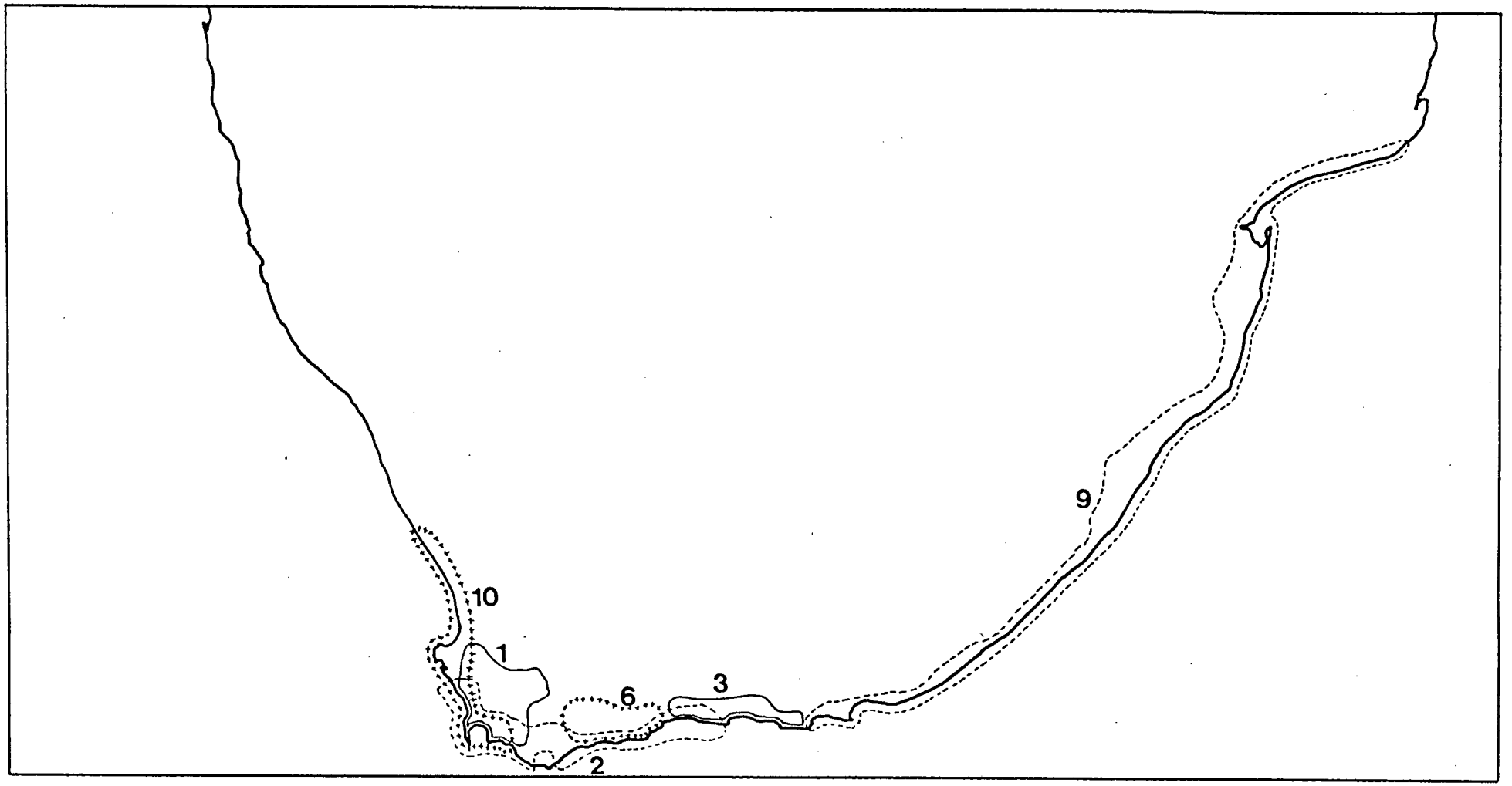


Figure 8: Distribution range outlines for 1 - *C.mon.mon.*; 2 - *C.mon.flo.*F1; 3 - *C.mon.flo.*F2; 6 - *C.mon.pis.ang.*; 9 - *C.mon.rot.*; 10 - *C.inc.inc.* Taxa occur in coastal vegetation where dotted lines cross terrestrial-aquatic boundaries (see Table 1b for abbreviations of taxon names).

3.2.4. Distributional Overlap Between Taxa

Distributional overlap between subspecies and varieties is given in Table 11.

Table 11: Percentage values for degree of distributional overlap between taxa of *Chrysanthemoides* (see Table 1b for abbreviations of taxon names).

	1	2	3	4	5	6	7	8	9	10	11
1	1.0	5.4	0	0	14.9	0	0	4.9	0	0	12.9
2		1.0	1.9	2.0	0	2.3	2.9	2.7	0	0	3.9
3			1.0	5.5	0	14.3	2.5	5.1	0	4.3	0
4				1.0	0	2.3	0	1.4	0	2.3	0
5					1.0	2.9	4	2.1	0	0	5.9
6						1.0	10.3	1.3	0	16.2	0
7							1.0	5.1	0	7.1	0
8								1.0	0	9	5.1
9									1.0	0	1.9
10										1.0	0
11											1.0

1. *C.mon.pis*.F1
2. *C.inc.sub.*
3. *C.inc.inc.*
4. *C.inc.mic.*
5. *C.mon.flo*.F2
6. *C.mon.flo*.F1
7. *C.mon.pis.ang.*
8. *C.mon.pis*.F2
9. *C.mon.can.*
10. *C.mon.mon.*
11. *C.mon.rot.*

Subspecies and varieties with high percentage estimates for overlapping distributions, are listed below:

- *C.mon.pis*.F1 and *C.mon.flo*.F2
- *C.mon.rot.* and *C.mon.pis*.F1
- *C.mon.flo*.F1 and *C.inc.inc.*
- *C.mon.flo*.F2 and *C.mon.pis.ang.*
- *C.mon.mon.* and *C.mon.flo*.F1

3.3. Cladistics

Parsimony analysis using Hennig86 located seven equally parsimonious trees of l = 58, C = 0.48 and R = 0.70, while PAUP located 16 equally parsimonious trees. Branching patterns described were identical for PAUP and Hennig86. The strict consensus tree results in the loss of several nodes (Fig. 9). Successive weighting reduced the number of trees to seven, with consistency and retention indices of 0.63 and 0.83 respectively. Phylogeny of the Coreopsideae (Asteraceae) retrieved consistency indices of 0.63 (Ryding & Bremner 1992) and *Aster* (Asteraceae), 0.218 (Jones & Young 1983). Retention indices of 0.83 indicating that the seven trees located for *Chrysanthemoides* are fairly uniform and characters used, provide good support for the topology of the weighted tree shown in Fig. 10. Character weightings introduced are given in Table 12.

Table 12: Character weightings for phylogenetic analysis using Hennig86, where maximum weight is 10 and the minimum weight is 0.

Character Number	1	2	3	4	5	6	7	8	9	10	11	12
Weighting	0	5	3	2	4	1	10	2	3	1	4	1

Character Number	13	14	15	16	17	18	19	20	21	22	23
Weighting	0	1	3	3	1	10	3	10	10	10	10

Five clades were identified from the successively weighted tree (Fig. 10). Group 1 is characterized by the spinescent taxa which

include *C. incana* ssp. *incana* var. *incana*, var. *microphylla*, var. *hirsuta* R.C. Griffioen, var. *rangei*, var. *gracilis* R.C. Griffioen and ssp. *subcanescens*. Group 2 is characterized by the globose/subglobose drupaceous subspecies, *C. monilifera* ssp. *monilifera*. Group 3, a dry inland group is characterised by *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1 & 2), var. *borealis* and var. *angustifolia*. Group 4 consists of Afromontane ssp. *canescens* and ssp. *septentrionalis*. Group 5 consists of coastal, non-spinescent subspecies, ssp. *rotundata* and ssp. *floribunda* (form 1 & 2)).

The results of the bootstrap analysis are shown in Fig. 9. Values indicate relatively strong support for two groups, one comprising Afromontane subspecies, *C. monilifera* ssp. *septentrionalis* and ssp. *canescens* with a bootstrap value of 0.60, and the other *C. incana* with a bootstrap value of 0.87.

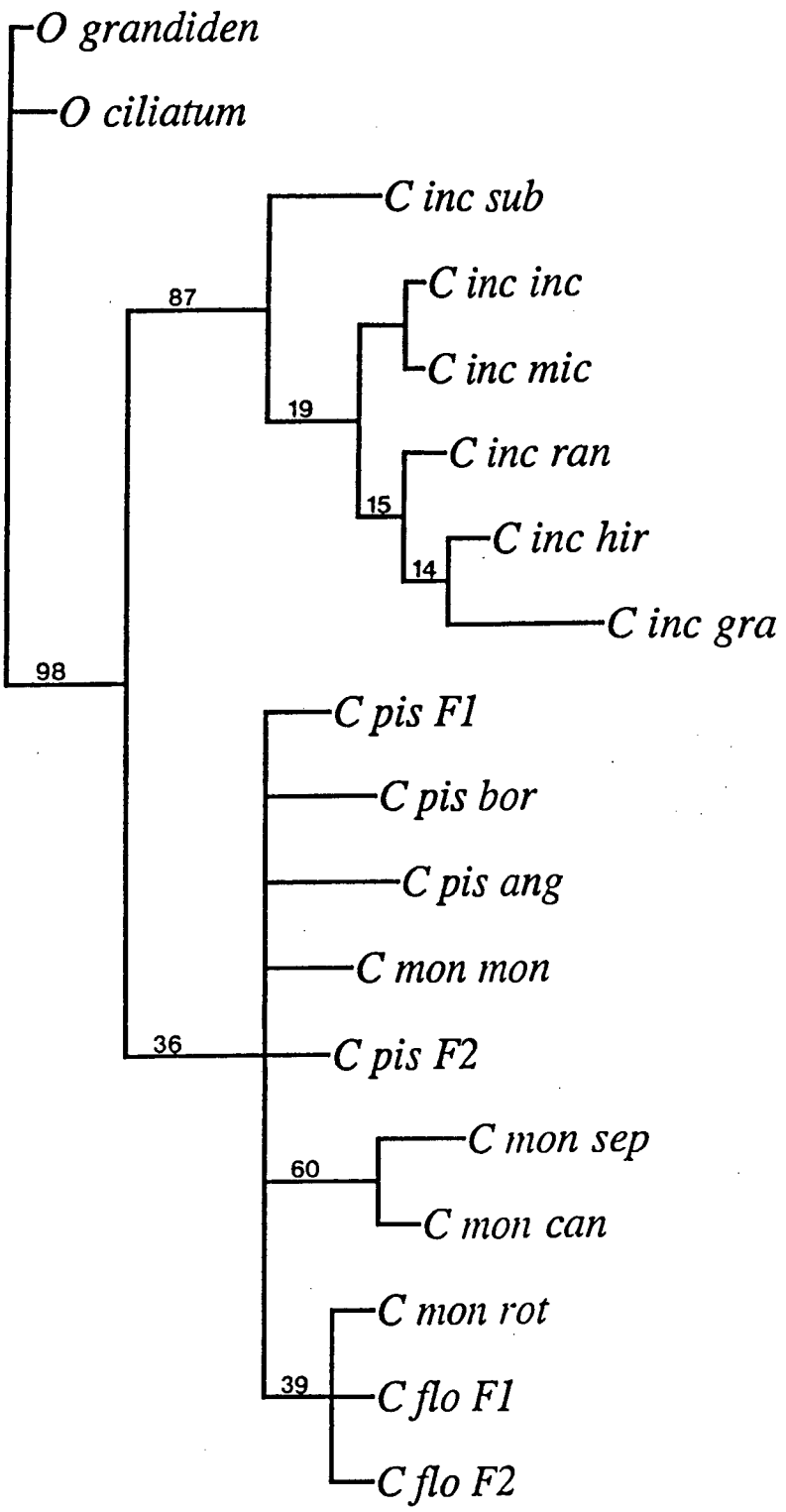


Figure 9: A strict consensus tree using nelsen option. Bootstrap values are included above branches (see Table 1b for abbreviations of taxon names).

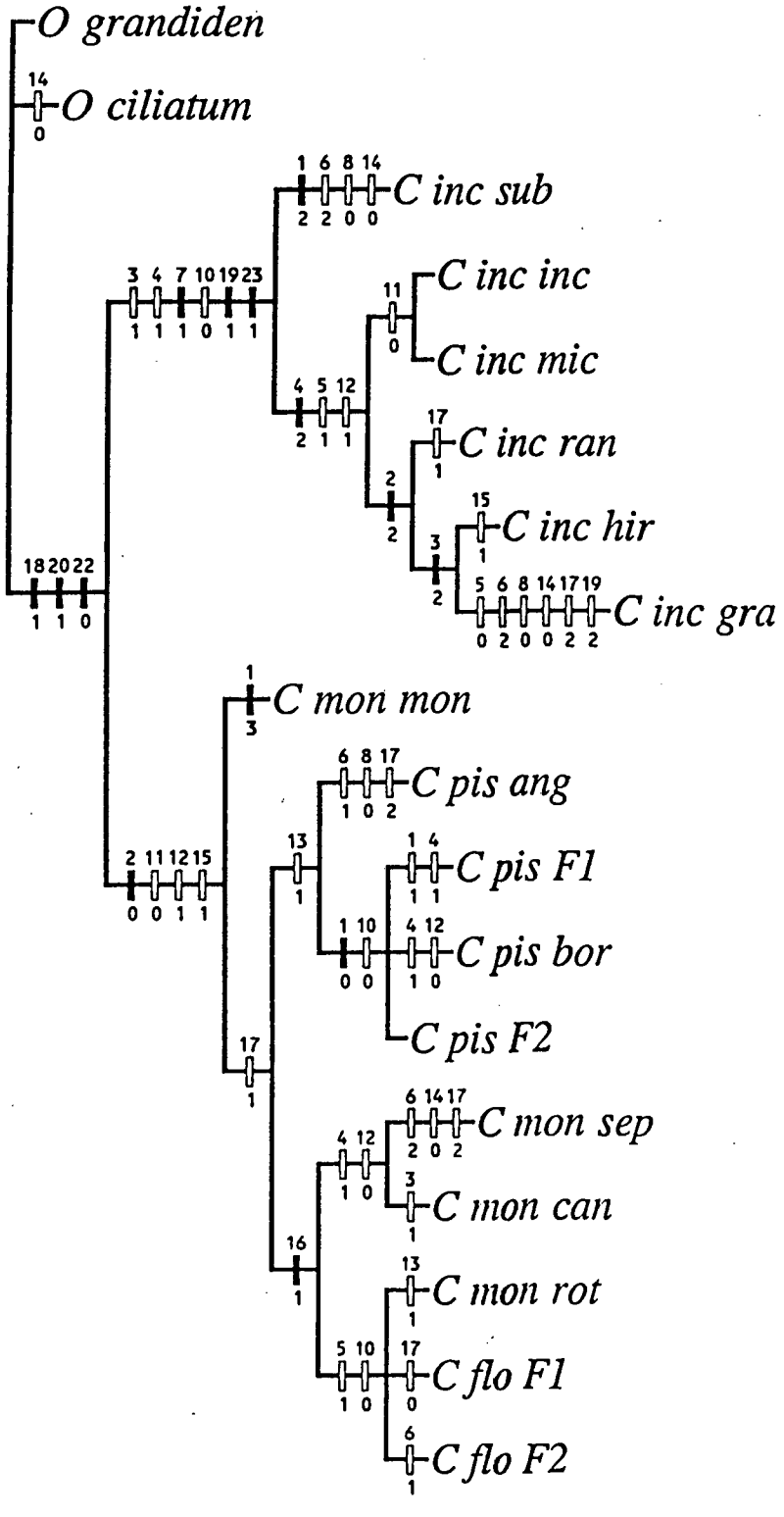


Figure 10: A successively weighted tree. Filled hash marks indicate non-homoplastic synapomorphies while empty hash marks indicate homoplastic state changes. Numbers above the boxes indicate characters while numbers below indicate character states (see Table 1b for abbreviations of taxon names).

Table 13: Data matrix used for the phylogenetic analysis. All characters were considered additive. Unknown or inapplicable states are coded with a question mark (?). See Appendix 3 for characters and character states and Table 1b for abbreviations of taxon names.

Taxon	Character number 1 1111111112 222 1234567890 1234567890 123
1. *O.grandiden.*	?100000111 1001??0020 010
2. *O.ciliatum*	?100000111 1000??0020 010
3. *C.inc.inc.*	1112101100 0101000111 ?01
4. *C.inc.hir.*	1222101100 1101100111 ?01
5. *C.inc.mic.*	1112101101 1101000111 ?01
6. *C.inc.ran.*	1212101100 1101001111 ?01
7. *C.inc.gra.*	1222021000 1100002121 ?01
8. *C.mon.sep.*	1001020121 0000112121 ?00
9. *C.mon.can.*	1011000121 0001111121 ?00
10. *C.inc.sub.*	1111021000 1000000111 ?01
11. *C.mon.pis.*F1	1001000100 0111101121 ?00
12. *C.mon.rot.*	1000100100 0111111121 ?00
13. *C.mon.flo.*F1	1000100100 0101110121 ?00
14. *C.mon.pis.bor.*	0001000110 0011101121 ?00
15. *C.mon.pis.ang.*	1000010011 0111102121 ?00
16. *C.mon.mon.*	0000000111 0101100121 ?00
17. *C.mon.pis.*F2	0000000110 0111101121 ?00
18. *C.mon.flo.*F2	1000110110 0101101121 ?00

3.4. Molecular Systematics

3.4.1. Isozymic Variation

Chrysanthemoides was found to be polymorphic at four loci (alpha-EST-2; ALD-2; GPH-2; beta-EST-2) and 15 loci coding for enzymes were partially resolved and identified. The percentage of polymorphic loci (P) averages for all populations, ranged from 46.6 to 66.7 %. The mean number of alleles per locus (A) ranged from 1.5 to 1.7 (S.E. = 0.100) and mean heterozygosity (H) ranged from 0.29 to 0.407 (S.E. = 0.072 - 0.122; HdyWbg expected) (Table 14).

Table 14: Mean number of alleles per locus (A), percentage of loci polymorphic (P) * and heterozygosity (H) using a direct count and Hardy-Weinberg expectations for *Chrysanthemoides*. Standard errors are given below values.

	(A)	(P)	(H)	
Form	Mean N^{o} of Alleles/Locus	% Loci Polymorphic*	Heterozygosity Direct Count	HdyWbg**
Still Bay	1.5	46.7	0.467	0.322
	(0.1)		(0.133)	(0.099)
Buffels Bay	1.6	60.0	0.600	0.366
	(0.4)		(0.131)	(0.085)
Hoekville	1.5	46.7	0.467	0.290
	(0.4)		(0.133)	(0.088)
Saldanha	1.7	66.7	0.667	0.381
	(0.3)		(0.126)	(0.072)
Skoenmakerskop	1.5	46.7	0.467	0.407
	(0.5)		(0.133)	(0.122)
Mean	1.56	53.36	0.534	0.353

* a loci is considered polymorphic if the frequency of the most common allele does not exceed 0.95
** unbiased estimate (Nei 1978)

3.4.2. Heterozygote Deficiency

Tables 15 - 19 show the results of the Chi-squared tests of deviations from Hardy-Weinberg expectations. Expected frequencies were calculated using Levene's (1949) formula for small sample size. Populations from Buffels Bay, Hoekville forest, and Saldanha show significant differences from the Hardy-Weinberg equilibrium, indicating that these three populations have high heterozygosity levels. Skoenmakerskop and Still Bay populations have lower levels of heterozygote deficiency but still indicate minimal inbreeding. Observed values do not equal individuals sampled (Table 15 - 18) because in many cases only a few of the individuals showed staining patterns.

Table 15: Observed and expected genotype frequencies for the Still Bay (*C. monilifera* ssp. *floribunda* (form 1)) population with a Chi-squared test for deviation from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size.

Loci	Class	Observed Frequency	Expected Frequency	Chi-Square	D.F.	P
EST-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
ADH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
PGM-1	A-A	0	0.86			
	A-B	5	2.29			
	B-B	0	0.86			
GPH-2	B-B	0	0.86			
	B-C	4	2.29			
	C-C	0	0.86			
ALD-1	A-A	0	0.00			
	A-B	1	1.00			
	B-B	0	0.00			
GDH-1	A-A	0	0.00			
	A-B	1	2.29			
	B-B	0	0.86			
SOD-1	A-A	0	0.00			
	A-B	1	1.00			
	B-B	0	0.00			
Mean				2.429	1	0.334

Table 16: Observed and expected genotype frequencies for the Buffels Bay population (*C. monilifera* ssp. *floribunda* (form 1)), with a Chi-squared test for deviations from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size.

Loci	Class	Observed Frequency	Expected Frequency	Chi-Squared	D.F.	P
EST-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
ADH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
PGM-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
GPH-1	A-A	0	0.00			
	A-B	1	1.00			
	B-B	0	0.00			
GPH-2	B-B	0	0.86			
	B-C	4	2.29			
	C-C	0	0.86			
ALD-1	A-A	0	0.86			
	A-B	4	2.29			
	B-B	0	0.86			
GDH-1	A-A	0	0.86			
	A-B	5	2.78			
	B-B	0	1.11			
PGI-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
Mean				3.222	1	0.163

Table 17: Observed and expected genotype frequencies for the Hoekville forest population (*C. monilifera* ssp. *floribunda* (form 2)) with a Chi-squared test for deviations from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size.

Loci	Class	Observed Frequency	Expected Frequency	Chi-Squared	D.F.	P
EST-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
ADH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
PGM-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
ALD-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
GDH-1	A-A	0	0.00			
	A-B	1	1.00			
	B-B	0	1.11			
SOD-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
PGI-1	A-A	0	0.86			
	A-B	4	2.29			
	B-B	0	0.86			
Mean				3.286	1	0.188

Table 18: Observed and expected genotype frequencies for the Saldanha population (*C. incana* ssp. *incana* var. *incana*) with a Chi-squared test for deviations from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size.

Loci	Class	Observed Frequency	Expected Frequency	Chi-Squared	D.F.	P
EST-1	B-B	0	1.11			
	B-C	5	2.78			
	C-C	0	1.11			
ADH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
PGM-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
GPH-2	B-B	0	1.11			
	B-C	5	2.78			
	C-C	0	1.11			
ALD-1	A-A	0	0.33			
	A-B	2	1.33			
	B-B	0	0.33			
GDH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
SOD-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
BET-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
BET-2	C-C	0	0.60			
	C-D	3	1.80			
	D-D	0	0.60			
PGI-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
Mean				3.500	1	0.084

Table 19: Observed and expected genotype frequencies for the Skoenmakerskop population (*C. monilifera* ssp. *rotundata*) with a Chi-squared test for deviation from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size.

Loci	Class	Observed Frequency	Expected Frequency	Chi-Squared	D.F.	P
EST-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
GDH-1	A-A	0	1.11			
	A-B	5	2.78			
	B-B	0	1.11			
Mean				1.143	1	0.727

Coefficients of heterozygote deficiency are given in Table 20. Results indicate that no heterozygote deficiency is evident. This is borne out by positive D values.

Table 20: Coefficients for heterozygote deficiency or excess for five *Chrysanthemoides* populations at their different loci.

Form	Loci	Obs. Het.	Exp. Het.	F_{IS}	D
Still Bay	EST-1	5	2.78	-0.80	
	ADH-1	5	2.78	-0.80	
	PGM-1	4	2.29	-0.75	
	GPH-2	4	2.29	-0.75	
	GDH-1	4	2.29	-0.75	0.77
Buffels Bay	EST-1	5	2.78	-0.80	
	ADH-1	5	2.78	-0.80	
	PGM-1	5	2.78	-0.80	
	GPH-2	4	2.29	-0.75	
	ALD-1	4	2.29	-0.75	
	GDH-1	4	2.29	-0.75	
	SOD-1	5	2.78	-0.80	
	PGI-1	5	2.78	-0.80	0.78
Hoekville Forest	EST-1	5	2.78	-0.80	
	ADH-1	5	2.78	-0.80	
	PGM-1	5	2.78	-0.80	
	ALD-1	5	2.78	-0.80	
	SOD-1	5	2.78	-0.80	
	PGI-1	4	2.29	-0.75	0.79
Saldanha	EST-1	5	2.78	-0.80	
	ADH-1	5	2.78	-0.80	
	PGM-1	5	2.78	-0.80	
	GPH-2	5	2.78	-0.80	
	ALD-1	2	1.33	-0.50	
	GDH-1	5	2.78	-0.80	
	SOD-1	5	2.78	-0.80	
	BET-1	5	2.78	-0.80	
	BET-2	3	1.80	-0.67	
	PGI-1	5	2.78	-0.80	0.76
Skoen-makers-kop	EST-1	5	2.78	-0.80	
	GDH-1	5	2.78	-0.80	0.80

D = ([Het. Obs./Het. Exp.]-1) (Wright (1965) fixation test)
F_{IS} = overall inbreeding coefficient of individuals including the effect of non-random mating and random genetic drift.

3.4.3. Genetic Distance and Similarity

Values for genetic similarity using Nei's (1972, 1978) genetic identity are given in Tables 21 to 22. Overall similarity for the five populations is given in Table 23.

Table 21: Genetic similarity using Nei's (1978) unbiased genetic identity for the five *Chrysanthemoides* populations.

Population	1	2	3	4	5
1 Still Bay	1.000				
2 Buffels Bay	0.804	1.000			
3 Hoekville	0.761	0.947	1.000		
4 Saldanha	0.785	0.882	0.835	1.000	
5 Skoenmakers-kop	0.776	0.726	0.754	0.705	1.000

Table 22: Genetic similarity using Nei's (1972) genetic identity for the five *Chrysanthemoides* populations.

Population	1	2	3	4	5
1 Still Bay	1.000				
2 Buffels Bay	0.774	1.000			
3 Hoekville	0.739	0.910	1.000		
4 Saldanha	0.746	0.830	0.793	1.000	
5 Skoenmakers-kop	0.761	0.705	0.739	0.676	1.000

Table 23: Genetic similarity for the five *Chrysanthemoides* populations

Population	1	2	3	4	5
1 Still Bay	1.000				
2 Buffels Bay	0.783	1.000			
3 Hoekville	0.783	0.947	1.000		
4 Saldanha	0.783	0.858	0.858	1.000	
5 Skoenmakers-kop	0.740	0.740	0.740	0.740	1.000

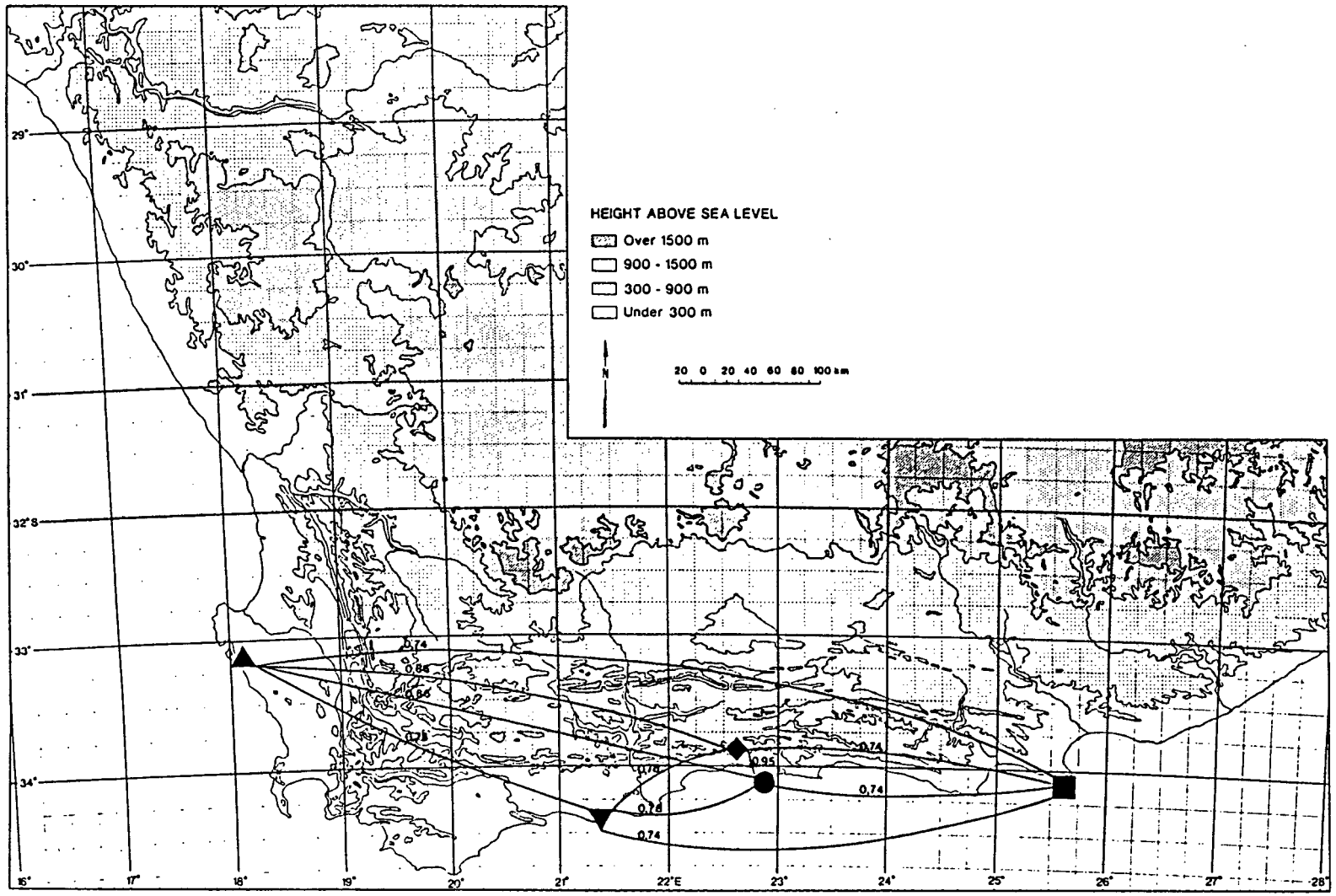


Figure 11: Localities and genetic similarity values between populations electrophoretically tested. ▲ - Saldanha (*C.inc.inc.*); ▼ - Still Bay (*C.mon.flo.*F1); ♦ - Hoekville (*C.mon.flo.*F2); ● - Buffels Bay (*C.mon.flo.*F1); ■ - Skoenmakerskop (*C.mon.rot.*). See Table 1b for abbreviations of taxon names.

Genetic distances are reflected in a phenogram produced by cluster analysis using the unweighted pair group method (UPGMA) (Fig. 12). The high cophenetic correlation coefficient (0.948), indicates that the phenogram is a good representation of the original distance matrix.

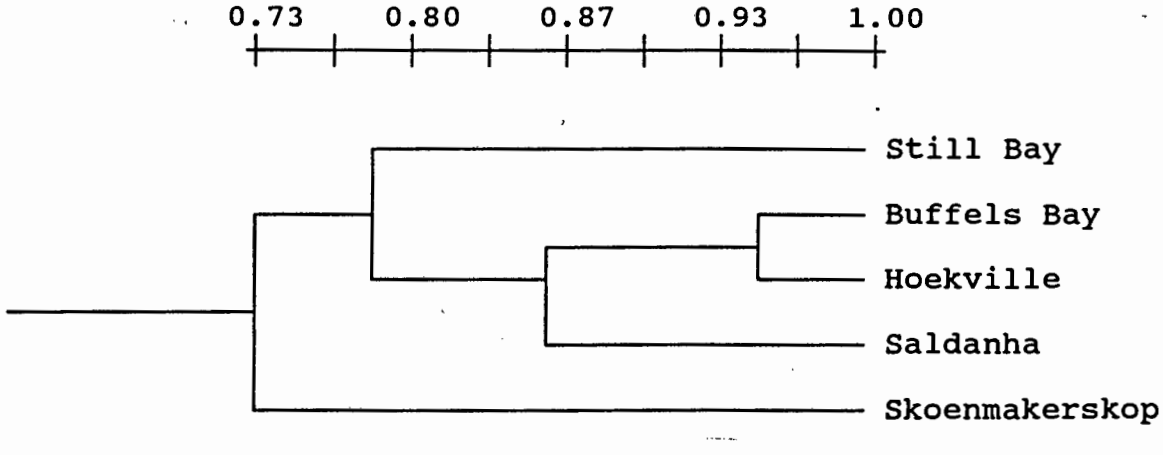


Fig. 12: Cluster analysis for the five *Chrysanthemoides* populations. A simmilarity axis is included. Still Bay - *C.mon.flo*.F1; Buffels Bay - *C.mon.flo*.F1; Hoekville - *C.mon.flo*.F2; Saldanha - *C.inc.inc.*; Skoenmakerskop - *C.mon.rot*. (see Table 1b for abbreviations of taxon names).

3.4.4. Electrophoretic Analysis of *Chrysanthemoides* Populations from Swellendam and Infanta.

Allele frequencies for the three populations collected in the Bredasdorp district are given in Table 24. All six loci stained for the three populations tested (Fig. 13). According to genetic similarity values (Table 25), populations are genetically identical for the enzymes tested. No cluster analysis was computed as a result. The percentage of polymorphic loci (P) for all populations was 50 %. The mean number of alleles per locus (A) was 1.5 (S.E. = 0.200) and mean heterozygosity (H) ranged from 0.273 to 0.277 (S.E. = 0.122 - 0.124; HdyWbg expected).

Table 24: Allele frequencies for the three populations collected from the Bredasdorp district. Population 1 - Swellendam (*C.mon.pis.ang.*); Population 2 - Malgas road (putative hybrid); Population 3 - Infanta (*C.mon.flo.*F1). See Table 1b for abbreviations of taxon names.

	Population		
Locus	1	2	3
EST-1 (N)	5	5	5
A	0.6	0.7	0.6
B	0.4	0.3	0.4
EST-2 (N)	6	4	4
A	0.0	0.0	0.0
B	1.0	1.0	1.0
PGI-1 (N)	4	4	4
A	0.5	0.5	0.5
B	0.5	0.5	0.5
LAP-1 (N)	5	5	5
A	1.0	1.0	1.0
MDH-1 (N)	5	6	6
A	0.5	0.5	0.5
B	0.5	0.5	0.5
MDH-2 (N)	4	4	5
A	1.0	1.0	1.0

Table 25: Genetic similarity values using Nei's (1972) genetic identity for Swellendam, putative hybrid and Infanta populations.

Populations	1	2	3
Swellendam	1.000		
Putative Hybrid	1.000	1.000	
Infanta	1.000	1.000	1.000

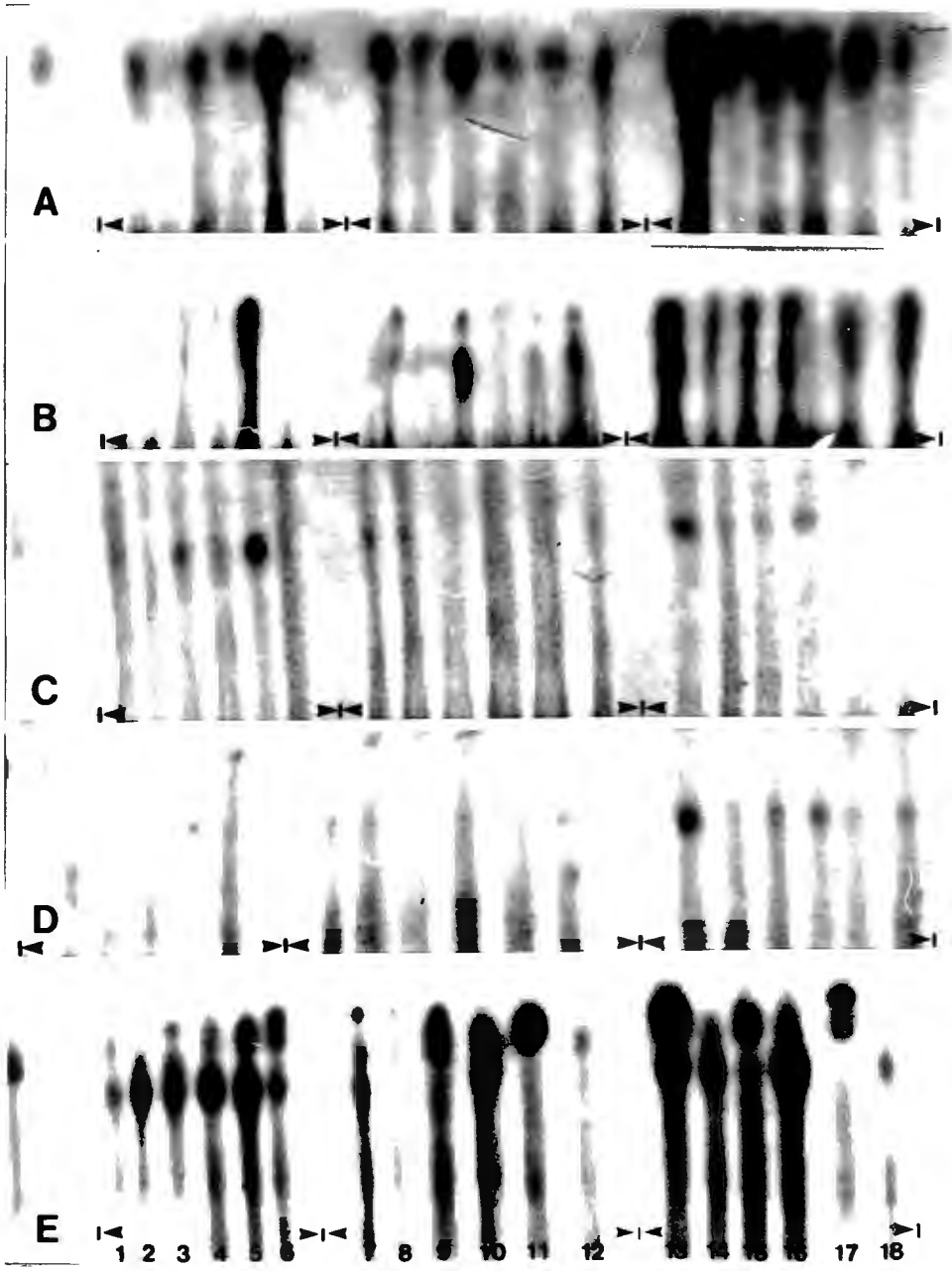


Figure 18: Electrophoretic phenotypes for *C. monilifera* ssp. *pisifera* var. *angustifolia* (1 - 6), a putative hybrid (7 - 12) and *C. monilifera* ssp. *floribunda* (form 1) (13 - 18). A). MDH using tray buffer system A (see appendix 4). B). MDH using tray buffer system C. All individuals are heterozygous for the faster migrating allozyme but homozygous at the slower migrating allozyme. C). LAP using tray buffer system A. D). LAP using tray buffer system C. All individuals are homozygous although staining patterns are not that distinct in the photograph. E). EST. Two loci stained for the allozyme. Individuals of population 1 are heterozygous for lanes 1 and 3 - 6 (faster migrating) and homozygous (slower migrating). Individuals for population 2 are heterozygous at lanes 9 - 11, homozygous (AA) at lanes 7 and 8, and homozygous (BB) at lanes 12 (faster migrating). Individuals for population 2 are homozygous at 4 lanes (slower migrating). Individuals for population 3 are heterozygous at lanes 13 and 15 to 17, and homozygous at lanes 18 (faster migrating). The slower migrating enzyme is homozygous at lanes 13, 14, 16 and 18.

4.0. Discussion

4.1. Numerical Phenetics

4.1.1. The PCA and Cluster Analyses

4.1.1.1. *Chrysanthemoides incana* ssp. *incana*.

The cluster analysis (Fig. 1) retrieved two clusters, which largely agree with *C. incana* and *C. monilifera*. The PCA (Fig. 3) corroborates the distinctness of *C. incana* from *C. monilifera*. *C. incana* ssp. *subcanescens* is marginally different from *C. incana* ssp. *incana* var. *incana* and var. *microphylla*, as suggested by PCA.

Characters that are diagnostic for *C. incana* include spinescence, prostrate growth form, distribution of pubescence on stems, leaves and receptacles and pungent branch terminals. Furthermore, the species has distinct geographical and ecological attributes, occurring along the dry west coast and the dry interior from Clanwilliam to Port Elizabeth. The taxon should therefore continue to be recognized as a distinct species from *C. monilifera*.

Multivariate methods identified several clusters within *C. incana*. *C. incana* ssp. *subcanescens* (Cluster 3), a subspecies with glabrous stems, leaves and inflorescences, separates from *C. incana* ssp. *incana* (Clusters 1 & 2) on the basis of the absence of pubescence on stems, leaves and inflorescences. Differences between clusters 1 and 2 occur in leaf size and pubescence distribution, with var. *incana* having obovate leaves larger than 20 mm and pubescence peeling from leaf surfaces while var. *microphylla* has obovate leaves less than 20 mm long, and stems, leaves and receptacles are usually covered with a white-grey pubescence. A group of 3 OTU's which clusters within the latter variety in the cluster analysis is *C. incana* ssp. *incana* var. *rangei*. Plants of this variety have a prostrate growth habit

and the obovate leaves are clustered at branch terminals. *C. incana* ssp. *incana* clustered above the 0.75 phenon line (Fig. 1).

Norlindh (1943) included *C. incana* ssp. *subcanescens* in *C. monilifera* despite the spinescent branch tips. Spinescence is a stable character unlike pubescence which may be environmentally influenced, and varies for coastal and inland plants. Numerical data (Fig. 1; 3), eco-geographical data (Tables 7 - 11), and the phylogenetic pattern (Fig. 9) suggest that ssp. *subcanescens* should be included into *C. incana*.

Geographical distribution (Section 5.0) and morphological variation reveal that *C. incana* ssp. *incana* may be further subdivided into a number of eco-geographical varieties not retrieved by PCA and the cluster analyses (Figs. 1 - 4). However, phylogenetic data (Fig. 9) was supportive of the eco-geographical subdivision separating ssp. *incana* into five terminals.

Isolated populations of *C. incana* ssp. *incana* var. *hirsuta* may be found on coastal dunes in the vicinity of Cape Agulhas in the Western Cape. Plants are prostrate, not higher than 0.5 m, and entirely pubescent. *C. incana* ssp. *incana* var. *incana* populations occur at Hermanus, Gans Bay and Gouritz River Mouth, up to 60 km from Cape Agulhas. Plants of this variety form larger shrubs, and leaves, stems and flower receptacles are partially pubescent or with a peeling pubescence.

C. incana ssp. *incana* var. *rangei* occurs from Port Nolloth in the Northern Cape, along the West Coast to Spencer Bay in Namibia. Populations have obovate leaves which are clustered at branch terminals. Plants are prostrate, not higher than 0.5 m. The described area is continuously exposed to coastal fog and soil conditions are sandy with a low nutrient content.

The area between Hondeklip Bay, Vanrhynsdorp and Port Nolloth seems to be uninhabited by *C. incana* populations. However, further south a slender leaf form, var. *gracilis*, occurs in the

vicinity of Clanwilliam, Calvinia and Hondeklip Bay. Plants are morphologically separable from other *C. incana* ssp. *incana* populations by their narrowly elliptic leaves which are entire and pubescent.

4.1.1.2. *C. monilifera* ssp. *septentrionalis* and ssp. *canescens*.

C. monilifera ssp. *canescens* clusters with ssp. *septentrionalis* in the phenogram (Fig. 1). The distinctness of ssp. *septentrionalis* and ssp. *canescens* is corroborated by PCA (Fig. 3). *C. monilifera* ssp. *canescens* differs primarily from the latter in having dentate leaf margins, pubescence which covers the stem surfaces and partially covers the leaf surfaces. Both subspecies are montane, occurring further north from the centre of diversification of *Chrysanthemoides* in the Western Cape (Maps 1 - 13).

4.1.1.3. *C. monilifera* ssp. *floribunda* (forms 1 and 2) and ssp. *rotundata*

C. monilifera ssp. *floribunda* forms 1 and 2 cluster separately in the phenogram (Fig. 1) and their distinctness is corroborated by PCA (Fig. 3). *C. monilifera* ssp. *floribunda* (form 2) has elliptic leaves with scolloped leaf margins. Involucral scales are ovate and covered with a fine pubescence. Young leaves and inflorescences are clustered at branch terminals, like that of ssp. *floribunda* (form 1) and ssp. *rotundata*. The distribution range of ssp. *floribunda* (form 2) is continuous with that of ssp. *floribunda* (form 1), and genetic identity values (Table 23) suggests that form 2 has a very similar genetic constitution to the latter. Numerical data suggest that ssp. *floribunda* (forms 1 & 2) are possibly different subspecies or varieties: however, genetic identity values (Table 23) suggests that form 2 is an ecotype of form 1.

C. monilifera ssp. *rotundata*, also a coastal subspecies, clusters separately from the rest of *C. monilifera* in the cluster analysis (Fig. 1). However, PCA (Fig. 3) suggests that the subspecies has similar morphology to ssp. *floribunda* (form 1). The PCA results are a better indication of morphological similarity when interpreted in conjunction with eco-geographical data. Both are coastal subspecies occurring in sandy dune soils, however, their distributions are allopatric and rainfall averages per annum are higher where ssp. *rotundata* occurs. *C. monilifera* ssp. *floribunda* (form 1) and ssp. *rotundata* have obovate to elliptic leaves, with young leaves clustered at branch terminals and covered in a hairy pubescence. *C. monilifera* ssp. *rotundata* generally has larger, more obovate leaves than those of ssp. *floribunda* (form 1).

4.1.1.4. *C. monilifera* ssp. *pisifera* complex and ssp. *monilifera*

The cluster analysis (Fig. 2), with better sampling, retrieved four clusters and some intermediates within cluster 6. The ordination (Fig. 4) supports the recognition of four subgroups within group six (Fig. 1). The PCA and cluster analysis (Figs. 2 & 4) indicate that a search for concordant characters is important. In cases where gaps in character distribution occur, the complex should be addressed to identify taxa, whether it be at the species, subspecies or variety level.

C. monilifera ssp. *pisifera* var. *angustifolia* (6a) has slender, elliptic leaves, and inner and outer involucral scales are ovate. Leaf margins are scolloped unlike the dentate leaf margins of var. *pisifera* (form 1) (6b) and var. *pisifera* (form 2) (6c). All three varieties have elliptic or ovate drupes. *C. monilifera* ssp. *monilifera* (6d) has globose or sub-globose drupes, and leaf shape and margins are the same as in cluster 6b and 6c. The varieties within the *C. monilifera* ssp. *pisifera* complex, cluster above the 0.75 phenon line.

4.2. Phytogeography and Ecology (Maps 1 - 13)

Habitat parameters are frequently used to separate species (Raven & Raven 1976; Linder 1980, 1990; Jonsell & Jonsell 1984; Andersson 1986). However, the ecological species concept (EcSC) (Van Valen 1976; Andersson 1990) has resulted in much controversy in delimitating species (Bremer & Erikson 1992).

The delimitation of taxa using phytogeographical and ecological data alone would not be a perfect criterion. The best results will be obtained rather by using both of these criteria along with phenetic and cladistic evidence, as well as genetic measures of *Chrysanthemoides* relationships.

4.2.1. The PCA of Ecological Variables

In *Chrysanthemoides*, a replacement sequence of morphologically varying taxa is suggested for ecologically varying habitats. Morphologically discrete groups - varieties, forms and populations with distinct ecological parameters - exist which may be grouped according to niche occupation. The scoring of ecological parameters such as soil and vegetation type in which taxa occur, poses a problem, since in many cases taxa occur in a variety of soil and vegetation types. The PCA presented in Fig. 5 represents taxa that cluster for altitude, rainfall and rainfall season. However, these ecological parameters scored are averages for each taxon. Habitat shifts of taxa will therefore not be picked up on PCA.

Cluster 1: *C. monilifera* ssp. *canescens* (7) and *C. monilifera* ssp. *septentrionalis* (8).

C. monilifera ssp. *canescens* and *C. monilifera* ssp. *septentrionalis* cluster together in PCA (Fig. 7), occupying similar altitudinal and rainfall ranges (Fig. 5). However,

distributions of the two subspecies are parapatric with no overlap (Fig. 6). Intermediate morphology (*Brass* 16649; *Chapman* 8037; *Muller* 2179) at their geographical interface suggests that these subspecies have the potential to co-occur, but competitive exclusion may have selected against morphological intermediates and prevented the co-occurence of populations.

The distribution of the two subspecies could be limited by altitude and temperature. *C. monilifera* ssp. *canescens* when growing at lower altitudes, morphologically resembles ssp. *septentrionalis*: pubescence on stems and leaves decreases and leaf margins become inconspicuously dentate.

Cluster 2: Xeromorphic Subspecies and Varieties

C. incana ssp. *incana* var. *rangei* (13), var. *gracilis* (14) and *C. monilifera* ssp. *pisfera* var. *borealis* (5) have allopatric distributions (Fig. 7). However, *C. incana* ssp. *incana* var. *microphylla* (11), *C. incana* ssp. *subcanescens* (15) and *C. monilifera* ssp. *pisifera* var. *pisifera* forms 1 and 2 (4) are partly sympatric. Where distributional overlap occurs between two taxa **(ssp. *subcanescens* and var. *pisifera* form 2)**, different ecological niches may keep the two distinct.

C. incana* ssp. *incana* var. *microphylla* and *C. incana* ssp. *subcanescens - The PCA (Fig. 5) and distribution map (Fig. 7) suggest that var. *microphylla* and ssp. *subcanescens* have eco-geographical similarities. *C. incana* ssp. *incana* var. *microphylla* has mostly coastal distributions but has been recorded further inland. *C. incana* ssp. *subcanescens* occurs in more arid areas and is distributed from Clanwilliam west, to Port Elizabeth (Swartkops River) along dry water courses. Morphological intermediates have been collected from Laingsburg and Bredasdorp. Specimens are slightly pubescent on their stem and leaf material, and branch terminals are conspicuously spinescent (*Compton* 2506; *Goldblatt* 1457; *O'Callaghan* 629; *Pillans* 12689).

Cluster 3: Cape Peninsula and Coastal Taxa.

***C. monilifera* ssp. *floribunda* (form 1) (2) and *C. incana* ssp. *incana* var. *incana* (10)** often co-occur (distribution overlap - 14.3 %), and in many cases are syntopic, but usually *C. monilifera* ssp. *floribunda* (form 1) occurs on coastal dunes and in amongst coastal vegetation while var. *incana* occurs in rocky, coastal vegetation. Both taxa occur further inland growing in deep sands of the Western Cape coastal plains alongside one another. *C. monilifera* ssp. *floribunda* (form 1) in the vicinity of Simonstown, Gans Bay and Hermanus resembles *C. incana* ssp. *incana* var. *incana*. However, plants are non-spinescent, although branch terminals are pungent. Leaves are small and plants have a stunted growth (*Hugo* 1685; *Oliver* 5112; *Stokoe* 60796).

The implications are that the two taxa are reproductively isolated, and so genetically distinct. The competitive interactions between the two forms are not fully understood since, if the taxa are reproductively isolated, competitive exclusion is not necessarily excluded. If ecological competition is taking place, the possibilities are that the two species are newly evolved and further divergence of their moisture and nutrient acquisition and other features associated with ecological competition are required.

Alternatively, the preferred habitat of *C. incana* ssp. *incana* var. *incana* is amongst coastal rocky vegetation where ssp. *floribunda* (form 1) has been excluded. The former species invades the ecological habitat of ssp. *floribunda* (form 1) but is continuously outcompeted. Sympatric populations could be remnant populations of two species competing, therefore neither of the species are excluded because of the different ecological habitat occupied.

***C. monilifera* ssp. *monilifera* (1) and *C. monilifera* ssp. *floribunda* (form 1) (2)** - morphologically intermediate populations have been collected at Rooiels and the Bredasdorp

district. Xerophytic features of ssp. *floribunda* (form 1) could be caused by high lime soil content and low soil moisture availability. A distribution overlap value of 16.2 % was calculated between the above two taxa (Table 9). Intermediate populations have obovate leaves which are arranged in terminal clusters (*Burger* 1372; *Lewis* 5139; *Oliver* 5112).

The two subspecies have different ecological requirements. Taxa separate altitudinally which may reflect a host of other environmental variables. *C. monilifera* ssp. *floribunda* (form 1) is predominantly a low altitude, coastal form whereas *C. monilifera* ssp. *monilifera* is usually montane.

Populations of ***C. monilifera* ssp. *monilifera* and *C. incana* ssp. *incana* var. *incana*** have been found to co-occur at the margins of their ecological requirements. Once again an altitudinal separation is suggested.

***C. monilifera* ssp. *floribunda* forms 1 and 2 (3), and *C. monilifera* ssp. *pisifera* var. *angustifolia* (6)** - the habitats of these taxa are compared in Fig. 8. Indications are that ssp. *floribunda* (form 1) and var. *angustifolia* partially co-occur. The former is a coastal form while var. *angustifolia* occurs inland on Bokkeveld shales on gentle mountain slope in the Langeberg vicinity. Field trips to the area revealed isolated populations with intermediate morphology. Sympatric populations of ssp. *floribunda* forms 1 and 2 occur in the vicinity of Knysna (Figs. 5 and 8). Fieldtrips to this area revealed that form 2 occurs along roadsides in the margins of forests while forms 1 is a coastal form inhabiting coastal dunes more in the open.

4.3. Cladistic Analysis

4.3.1. Cladistic Patterns and Bootstrap Values

The topology of the cladogram (Fig. 10) separated into two

genera, the outgroups *Osteospermum grandidentatum* and *Osteospermum ciliatum*, and the genus *Chrysanthemoides*. The monophyly of *Chrysanthemoides* was not tested in this analysis, but is supported by drupaceous fruits, a cobweb-like pubescence on stems, leaves and flower receptacles, and petiolate leaves. Other characters such as sterile disc florets and the absence of a pappus are plesiomorphic and do not demonstrate monophyly at the genus level. Pubescence on leaves, stems and receptacles as well as spinescent characters, have a strong overriding power in determining the outcome of the cladogram. A bootstrap index of 0.98 was calculated for *Chrysanthemoides*. Groups will be explained in terms of the character consistency for the taxa described.

The two basal clades located by the cladistic analysis (Fig. 10) are consistent with the two species retrieved by the multivariate analysis: 1) a strongly supported monophyletic group, *C. incana,* and 2) another presumably monophyletic but less well supported group, *C. monilifera*.

The *C. incana* group, which consists of seven varieties in two subspecies, is characterised by the non-homoplastic synapomorphies, spinescence and pungent branch terminals, and the homoplastic state changes include pubescent stems and receptacles, and leaf texture. A bootstrap index of 0.87 was calculated for the group. Within the *C. incana* group, there are two subspecific clades, ssp. *incana* and ssp. *subcanescens*. The former clade is characterized by pubescent receptacles, obovate leaves and absence of tannin in stem material. *C. incana* ssp. *subcanescens* is characterized by a high drupe length/drupe breadth ratio, entire or serrate leaf margins, narrow leaf widths and a low leaf width at widest outdent/leaf width at inner sinus ratio. *C. incana* ssp. *incana* divided into five rather vaguely characterized varieties: 1) var. *incana* and var. *microphylla* both characterized by an erect growth form, 2) var. *rangei* which has a high inner involucral scale/outer involucral scale ratio and pubescence on the adaxial surfaces of the leaves, 3) var. *hirsuta*

with a short petiole length and stem material completely covered in pubescence and, 4) var. *gracilis* having narrowly elliptic leaves, entire leaf margin, small marginal floret length/capitulum diameter ratio, a high inner involucral scale/outer involucral scale ratio and a low leaf width at widest outdent/leaf width at inner sinus ratio.

The larger subgroup of the two *Chrysanthemoides* species, *C. monilifera*, is a rather poorly supported monophyletic group of non-spinescent taxa. The subgroup is supported by an additional similarity between the taxa, absence of pubescence on the adaxial surfaces of the leaves, a non-homoplasious synapomorphous character. Tannin absence in stem material, erect growth form and high leaf length/leaf petiole length ratio further support the group. Within the *C. monilifera* assemblage there are three infraspecific groups:

C. monilifera ssp. *monilifera*, is the sister to the rest of the species, and consists of populations with the non-homoplastic synapomorphic character of globose/sub-globose drupes. The dentate leaf margins and absence of pubescence from stems, leaves and flower receptacles are also known from ssp. *pisifera*, and may be a case of convergence.

C. monilifera ssp. *pisifera*, is poorly supported by the presence of tannins in leaf material. The group splits into two lineages. *C. monilifera* ssp. *pisifera* var. *angustifolia* has scolloped leaf margins, narrow leaves and a low inner involucral scale/outer involucral scale ratio. The latter separated into two varieties and two forms characterized by high drupe length/drupe breadth ratios and leathery leaves. 1) var. *pisifera* (form 2) not characterized by any characters, 2) var. *pisifera* (form 1) having a high drupe ratio and pubescent receptacles and 3) var. *borealis* with pubescent receptacles and absence of tannins in the stem material. Bootstrap indices for the groups are low, ranging from 0.16 to 0.30.

The final clade consists of two groups, an Afromontane group and coastal group both characterized by drupe ridging. The Afromontane group is supported by stem tannins and pubescent flower receptacles. *C. monilifera* ssp. *canescens* is characterized by pubescent stems, while ssp. *septentrionalis* is supported by spinescent leaf margins, low inner involucral scale/outer involucral scale ratio and low leaf width at outdent/leaf width at inner sinus ratio. A bootstrap index of 0.60 gauged the strength of character support for the group. Coastal taxa are characterized by obovate leaves and leathery leaves. The clade splits into three subspecies: ssp. *rotundata*, supported by tannins in stem material, ssp. *floribunda* (form 1), supported by low inner involucral length/outer involucral scale length ratio; and ssp. *floribunda* (form 2) characterized by scolloped leaf margins. A bootstrap index of 0.39 was calculated for the group.

4.4. Molecular Techniques

4.4.1. Genetic Identity and Geography

Chrysanthemoides identity values do not always correlate with the geographical distance separating taxa (Fig. 11). Populations of *C. incana* ssp. *incana* var. *incana*, distributed along the Cape West Coast, have higher average identities than *C. monilifera* ssp. *rotundata* to the rest of the forms electrophoretically tested. The results suggest that *C. incana* ssp. *incana* var. *incana* has had a more recent contact with *C. monilifera* than *C. monilifera* ssp. *rotundata*, and that subspecies with the same morphology may not have similar genotypes.

Highest identity values were calculated between *C. monilifera* ssp. *floribunda* form 1 and 2, which are geographically isolated populations. High genetic identity values may also indicate gene flow between these populations. *Chrysanthemoides* genetic similarity data reveal that values calculated between parapatric, infraspecific taxa (ssp. *floribunda* form 1 and 2 from Buffels Bay

and Hoekville), are higher than those calculated between ssp. *floribunda* (form 1) from Still Bay and Buffels Bay. Thus the results correlate well with the notion that genetic identity and geographical separation are indirectly proportional.

Genetic identity values for the Bredasdorp complex (Fig. 13, Table 25) representing *C. monilifera* ssp. *pisifera* var. *angustifolia*, *C. monilifera* ssp. *floribunda* form 1 and an intermediate, are very high (1.000). The three populations tested are separated by less than 70 km, and multivariate (Figs. 1 - 4) and cladistic analysis (Figs. 9 - 10) suggest that *C. monilifera* ssp. *floribunda* (form 1) and ssp. *pisifera* var. *angustifolia* are morphologically distinct. Identity values for the above complex and between ssp. *floribunda* forms 1 and 2 (Table 23) suggest that populations within close vicinity of one another have regular events of gene exchange.

The low identity estimates for *C. monilifera* ssp. *rotundata* from Skoenmakerskop (0.740) to all other studied populations, may suggests a lengthy geographical isolation period from other *C. monilifera* populations. Only two out of the ten enzymes tested, stained for ssp. *rotundata* populations (EST-1; GDH). Explanations for staining patterns may be interpreted in terms of putative genetic differences which indicate that ssp. *rotundata* is a different subspecies. Bayer (1988) found that populations of *Antennaria marginata* and *A. rosulata* with high genetic identities (0.978), are morphologically very different (Bayer 1987) and their distribution ranges do not overlap. Heywood and Levin (1984) found that a significant correlation between genetic and geographical distance exists between populations of *Gaillardia pulchella*, suggesting that geographical isolation may have played a role in allozyme divergences for the two populations. However, allozymically similar populations are in some cases geographically remote. Crawford and Bayer (1981) found that genetic identity and distance separating two taxa are not always indirectly proportional. Similarly, *Chrysanthemoides* identity values do not necessarily decrease when analysing further up the

taxonomic hierarchy, and for allopatric populations. Therefore, enzymes that failed to stain for the Skoenmakerskop population in the electrophoretic experiments suggest that the grinding buffer system used in the analysis was incorrect. In the case of *C. incana* ssp. *incana* var. *incana* incorrect interpretation of starch gels could have biased genetic identity and distance values.

4.4.2. Genetic Identities Compared to Other Studies

The mean genetic identity values calculated between *Chrysanthemoides* populations in the initial electrophoretic experiment, are low ranging from 0.740 to 0.947, with an average identity of 0.795 (Table 23). These values indicate that populations are genetically similar, but not as close as one would have expected. Low estimates could be related to the geographical isolation of populations from one another.

Populations of *C. monilifera* ssp. *floribunda* (form 1) and ssp. *floribunda* (form 2) which were found in close vicinity of one another had genetic identity value ranging from 0.947 - 1.000. Genetic identity values calculated for populations between infraspecific taxa (subspecies and varieties), are generally similar for conspecific populations (Crawford 1983; Giannasi & Crawford 1986). Values calculated by Cosner and Crawford (1990) at the variety level (0.819), were equivalent to values calculated for *Chrysanthemoides*. High identity values are not surprising for infraspecific taxa, since they are usually recognized on the basis of morphological gaps although they are not necessarily reproductively isolated. Many recent studies have reported similar findings for conspecific genotypes (Gottlieb 1981; Crawford 1983). Cosner and Crawford (1990) reported genetic values of 0.934 for selfing and outcrossing plant populations. This indicates that genetic identity values for populations of *Chrysanthemoides* are within the correct range.

The two species were tested at ten different loci (experiment one), all the loci tested being heterozygous. Only eight loci for *C. monilifera* stained. Of these eight, seven were identical to that of *C. incana*. The remaining, GDH-2 did not stain in *C. incana*. Of the ten loci that stained for *C. incana*, B-EST-1, B-EST-2 and SOD-1 were not present in *C. monilifera*. Electrophoretic data indicates that the two populations possess different alleles and loci at 20 % of their enzymes tested, which is consistent with their recognition at specific rank.

Genetic identity estimates between Still Bay and Buffels Bay populations (0.783) are the same as those for Buffels Bay and Saldanha populations (Table 23), which may indicate that estimates are high at the congeneric level. Crawford and Whitkus (1988) report that in cases of rapid speciation, the species do not necessarily exhibit greater divergences than populations of the same species. Values calculated between *C. incana* and *C. monilifera*, ranged from 0.740 to 0.858 (Table 23). High values and morphological dissimilarity suggest recent but delayed divergence (Crawford 1983) for *C. monilifera* ssp. *floribunda* (form 1) and *C. incana* ssp. *incana* var. *incana*. Gottlieb (1977) gave a mean of 0.67 for species within the same genus from a variety of plant families. Lowrey and Crawford (1985) indicate that the mean genetic identity values for congeneric species, is much higher. Genetic identity values calculated for populations of different species and infraspecific taxa ranged from 0.87 to 1.00. Low identity values are characteristic of 'good' taxonomic species in plants examined electrophoretically (Lowrey & Crawford 1985).

4.4.3. Fit of Electrophoretic Results to Numerical Phenetics and Cladistics

Cluster analyses (Figs. 1 - 2), PCA's (Figs. 3 - 4) and cladograms (Figs. 9 - 10) suggest that *C. monilifera* and *C. incana* are separate species. Indications are that the two species

differ in many morphological features. Morphological interpretations in conjunction with electrophoretic results suggests that *C. monilifera* and *C. incana* should be classified as two separate species.

The molecular results, like the numerical and phylogentic results, indicate that the two taxa occurring in the Knysna district are very closely related. Thus the genetic similarities between populations indicate that any one population should be highly representative of the isozymic variation within the subspecies, since no barrier is preventing gene flow between populations. *C. monilifera* ssp. *floribunda* (form 2) is a biotype of ssp. *floribunda* (form 1), which has managed to expand its range into a light limited environment. Associated with this ecological change, was a morphological adaptation due to phenotypic plasticity. Similarily, neighbouring populations of the Swellendam and Infanta areas are morphologically distinct but genetically identical. Part of the success of *Chrysanthemoides* as a wide spread genus could be due to its ability to express its phenotypic plasticity in ecologically different areas.

4.4.4. Population Structure

The coefficient of inbreeding (F_{IS}) (Table 20) is useful for determining the reduction in heterozygosity due to non-random mating. Negative values for F_{IS} indicate outcrossing while positive values indicate inbreeding, and 0.00 is indicative of random mating. Values for Wright's fixation test (D) indicate the opposite to F_{IS} values. Negative F_{IS} and positive D values calculated for *Chrysanthemoides* indicate a high degree of outcrossing. All the populations tested electrophoretically have high levels of heterozygosity, since observed levels greatly exceed expected values. Bayer (1988) found similar results for *Antennaria*, a dioecious, perennial herb. His reason for high heterozygosity was survivorship of heterozygotes, and agamospermous seed production by heterozygous individuals.

Chrysanthemoides flowers are protandrous and the seeds are usually produced by cross fertilization (Weiss 1986). Bees and monkey beetles have been observed as pollinating agents but there are probably other pollinators as well. Drupes of *Chrysanthemoides* are edible, mainly eaten by birds (Knight 1988). Estimates of seedling production per plant are some 287 seedlings after each fruiting season. There is the possibility that the Compositae are basically self-incompatible (Burtt 1977) which supports the notion that *Chrysanthemoides* is largely outcrossed. High heterozygosity estimates for *Chrysanthemoides* may be interpreted in terms of wide seed dispersal, the large number of seedlings produced and the self-incompatibility of capitula. Futhermore, high heterozygosity could indicate that no sexual reproduction is taking place at all since sexual reproduction produces only heterozygotes. Perhaps populations of *Chrysanthemoides* consisted of individuals vegetatively reproduce by means of underground runners which have been observed in *C. incana* ssp. *incana*. Other explanations for high heterozygosity could be inadequate sampling which severely biased heterozygosity estimates.

Genetic divergence among and within populations is high, revealed by the genetic statistics in Table 14. The average number of alleles for the five populations is 1.56, higher than the 1.26 calculated by Bayer (1988) for plants with perennial, dioecious life-history traits. Mean values for number of alleles (A) were similar to mean values of 1.32, reported by Bayer (1988) and 1.00 to 1.76 by Rieseberg *et al.* (1991). The percentage polymorphic loci (P) for *Chrysanthemoides* was 53.36 %, which is higher than 36.8 % given for outcrossing species (Hamrick *et al.* 1979). Rieseberg *et al.* (1991) reports values for percentage of polymorphic loci, which range from 0.0 to 58.8 % for *Helianthus* populations. Mean values of heterozygosity (H) for *Chrysanthemoides* (H_{obs} = 0.534) were above average when compared to values calculated for *Helianthus* (H = 0 - 0.146 % (Rieseberg *et al.* 1991)) and other outcrossing plant species (H = 0.065 % (Bayer 1988)). In a review of life history characteristics and

electrophoretically detectable variation in plants (Hamrick *et al.* 1979), high heterozygosity estimates ranging from 0.407 to 0.481 are given for nine out of 113 species. The results indicated that plants populations were all produced by seed, outcrossed by sexual reproduction. Plants were short or long lived perennials. *Chrysanthemoides* has the same life history characteristics to the species described above.

Chrysanthemoides taxa displayed high levels of genetic variability, when compared with outcrossing populations of plants (Bayer 1988; Rieseberg *et al.* 1991). Mean values for P, A and H where higher than mean values reported by Hamrick *et al.* (1979). The isozyme electrophoresis completed for *Chrysanthemoides* indicates that populations tested in experiment 1 are reproductively isolated (Fig. 11), except for *C. monilifera* ssp. *floribunda* forms 1 and 2. When the data was analyzed in association with distribution, populations within reproductive distance were less genetically variable than widespread populations. Hamrick *et al.* (1979) states that the presence of a large number of locally adapted ecotypes could lead to the maintenance of large amounts of genetic variation within plant populations. Furthermore, perennials are less likely to be affected by genetic drift and thus maintain higher levels of genetic variability. Long-lived perennial such as *Chrysanthemoides* often consist of representatives from a number of generations. These generations would maintain genetic variability within populations.

4.4.5. Adequacy of Isozyme Electrophoretic Results

The isozyme electrophoretic section is weakened in that calculations for population genetic statistics, proportion of polymorphic loci, number of alleles per locus and heterozygosity estimates are severely limited by the small sample size used. Population sample size ranged from 5 - 7 individuals per population tested. Coefficients of population differentiation and

fixation indices cannot be meaningfully interpreted with such small sample size.

If sample size is small a measure of variance (standard error) is essential to meaningfully interpreted the data. BIOSYS-pc (Swofford & Selander 1989) does not give any measure of confidence for genetic similarity and distance values. Standard errors applied to the mean number of alleles and observed and expected heterozygosity estimates, may be used to judge the appropriateness and value of the findings. Standard error indicates low variability for mean number of alleles and heterozygosity, suggesting that the findings are an accurate representation for the individuals of the population sampled. However, the small sample size used could be a small reflection of the whole population making the analysis inadequately sufficient.

Interpretation of gel banding patterns also proved to be difficult since no previous isozyme electrophoretic studies on *Chrysanthemoides* exists and knowledge of enzyme subunit structure and genetic control of the enzyme system is limited. Resolution of banding patterns on zymograms was poor effecting the interpretation of the numerical analysis. Furthermore, only two buffer systems were used to asses banding patterns which failed to separate isozymes fully. Of the two isozyme electrophoretic analyses the first identified 15 different loci while the second only six. The number of loci identified in the second analysis is inadequate which may severely impinge on the interpretation of the analysis.

4.5. Criteria for Assignment of Taxonomic Rank

4.5.1. Species Concepts

There are several species concepts current in the literature (Grant 1971; Cracraft 1989; Templeton 1989; Baum 1992). These have been grouped as the biological species concept (Wallace 1889; Dobzhansky 1937, 1970; Huxley 1942; Mayr 1942, 1963, 1969,

1991), the cohesion species concept (Templeton 1989) and the recognition species concept (Paterson 1981, 1982, 1985), all based on a 'process' definition (Nelson 1989). However, the evolutionary species concept (Simpson 1951, 1961; Meglitsch 1954), the ecological species concept (Van Valen 1976; Andersson 1990) and the phylogenetic species concept (Eldredge & Cracraft 1980; Nelson & Platnick 1981; Cracraft 1983, 1987, 1988; Nixon & Wheeler 1990; Wheeler & Nixon 1990; Vrana & Wheeler 1992), the latter which includes the concept of monophyly (Hennig 1966; Rosen 1978, 1979; de Queiroz & Donoghue 1988, 1990) are 'pattern' defined (Nelson 1989).

The biological species concept or isolation concept (BSC) is defined by Mayr (1969) as 'groups of interbreeding natural populations that are reproductively isolated from other such groups.' This definition implies that species constitute 'reproductive communities', the individuals of which recognize one another as reproductive mates for the purpose of producing offspring. Furthermore, species are 'ecological units' occupying a space in the environment distinct from those of other species. Lastly, species are 'genetic units' representing a distinct gene pool or genetic system, isolated from other such communities by reproductive barriers. Critical evaluations of the BSC are numerous and are widely discussed (Mayr 1957; Sokal & Crovello 1970; Cracraft 1989; Frost & Hillis 1990). The BSC has its principle flaw in the inability to deal with uniparental organisms. Uniparental organisms are not included in this concept and the argument is that if there are no breeding populations, there are no biological species (Grant 1971). Furthermore, the BSC fails to identify speciation events and therefore does not explain why reproductive barriers should evolve (Andersson 1990).

One situation where the BSC comes into conflict with morphological criteria, is where breeding relationships and morphological distinctions do not coincide, due to the 'leakiness' of species genetically (Grant 1971). In cases where the breeding relationships agree with modern methods of analysing

morphological discontinuity, biological and taxonomic species are identical. However, if this is not the case it is important to distinguish between biological species and taxonomic species (Cain 1954; Grant 1963).

The cohesion species concept (CSC) is defined by Templeton (1989) as 'the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms.' Species are defined in terms of genetic and morphological similarity (mechanisms generating recognition) rather than the unveiling of recognition over evolutionary time (EvSC). Species are defined as an evolutionary lineage through the mechanisms, such as genetic drift, natural selection and gene flow, that keep populations of species distinct. Furthermore, the CSC identifies the processes that clarify a 'good taxonomic species', species that are geographically and genetically distinct for long periods of time. Here, sympatric populations that remain genetically distinct implies that populations are genetically isolated. Coexistence of these 'good species' suggests that they are also ecologically distinct (Mayr 1970). A 'good species' is therefore isolated by both genetic and demographic exchangeability (Templeton 1989).

The recognition species concept (RSC) is defined by Paterson (1985) as 'that most inclusive population of individual biparental organisms which share a common fertilization mechanism.' He addresses species and speciation in animals, but neglects the RSC in plants. Species are defined by an 'effective fertilization mechanism' in animals. However, the application of the RSC to plants must consider the mediation of pollination between two plants via a pollinator. Paterson (1985) argues that isolating barriers (BSC) are irrelevant, since they are misleading when thinking about the processes of speciation. The BSC and RSC are the inverse of one another, the former addresses the barriers to gene flow between species, while the latter focuses on the positive functions of the mechanisms that facilitate reproduction. Therefore, when defining species and the

processes of speciation such that it facilitates the study of speciation as an evolutionary process, the RSC is superior to the BSC.

The evolutionary species concept (EvSC) is defined by Simpson (1961) as 'populations or groups of populations with an ancestral-descendant sequence existing in space and time which evolves separately from other species (lineages).' The lineage occupies its separate ecological niche in the biotic environment but may be susceptible to change in evolutionary role through time. The EvSC has the ability to define species on a biological basis common to uniparental and biparental organisms (Grant 1971). The concept has the advantage over other concepts because it holds species together through gene flow, developmental, ecological and genetic constraints, and may be applied to living and extinct populations of organisms. The EvSC does have flaws. The concept fails to explain why different lineages are morphologically different, and therefore fails to explain differences in diversities and reproductive strategies, and how species arise (Andersson 1990).

The phylogenetic species concept (PSC) is outlined as 'the least inclusive population or set of populations among which there is character-based evidence in the form of fixed differences, that gene exchange does not occur' (Davis and Nixon 1992). This concept is similar to the EvSC in that 'character-based evidence' for two individuals may represent a common evolutionary fate. The PSC does not state that gene flow occurs but implies that interbreeding between populations of different species does not occur. 'Minimal diagnosable species' are identified in this way through population aggregation analysis, a technique recognized for its rigorous approach to the study of cladistic patterns (Davis & Nixon 1992). The PSC requires that individuals belonging to a species carry the entire complement of characters of that species.

An outline of PSC and contributors to this field of thought is

given by Baum (1992). One of these concepts is the autapomorphic or monophyletic species concept described by de Queiroz and Donoghue (1988, 1990), and Hennig (1966). Species, like higher taxa are described as including all the descendants and their common ancestors, thus forming complete systems of common ancestry, also known as clades or monophyletic groups, and the products of evolution above the species level are groups composed of ancestral species and their descendants. He regards species as appropriate terminals for phylogenetic analysis since they are related to one another through common ancestry. His model assumes that relationships between terminals in a phylogenetic analysis are hierarchic and characters of ancestors are retained in the original or transformed state.

The ecological species concept (EcSC) of Van Valen (1976) is described as 'a lineage (or a closely related set of lineages) which occupy an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range.' A lineage is defined as a clone or ancestral-descendant sequence of populations. An adaptive zone is some part of the ecological hyperspace occupied by the group considered. A lineage is recognized on the basis of these 'ecological units'. Andersson (1990) states that the EcSC has the ability to explain speciation on a basis common to all organisms. Although ecological criteria cannot be used to define individual species, they may be used to decide whether or not to given taxonomic recognition to a phenotype where morphology is not conclusive.

4.5.2. Species

Chrysanthemoides can be separated into two species on the basis of the BSC. Field observations suggest that the two species are genetically distinct and reproductively isolated (Mayr 1942). Isozyme electrophoresis genetic similarity values (0.78) indicate that samples from the two populations are different genetically

which would be expected if their collecting sites were separated more than 450 km. *C. incana* and *C. monilifera* are distinct when co-occurring, however, intermediate individual are found at a limited number of areas where co-occurring populations are not found. The implications are that these intermediates are a result of phenotypic plasticity rather than hybridization between sympatric populations. *C. monilifera* ssp. *floribunda* (form 1) in the vicinity of Simonstown, Gans Bay and Hermanus resemble var. *incana*, however, plants are non-spinescent but branch terminals are pungent. Leaves are small and plants have a stunted growth (*Hugo* 1685; *Oliver* 5112; *Stokoe* 60796).

The EcSC may be used to separate the two species in coastal areas. *C. monilifera* ssp. *floribunda* (form 1) occurs on coastal dunes and in amongst coastal vegetation, while *C. incana* ssp. *incana* var. *incana* usually occurs amongst rocky, coastal vegetation. Here the two populations are sympatric but ecologically distinct and therefore can be referred to as 'good species' (Mayr 1970). However, both taxa co-occur further inland growing in deep sands of the Western Cape coastal plains. The implication is that the two species occupy the same ecological niche but are still genetically distinct. Here, the EcSC does not function, however, the CSC states that if populations remain genetically distinct for long periods of time, then they are genetically isolated (Templeton 1989). Western Cape populations of ssp. *floribunda* form 1 and var. *incana* are genetically distinct and implications are that they have been like this for a long time since no hybrids where found in sympatric areas.

The presence of fixed character differences identified in cladistic and phenetic analysis (spinescence; pungent branch terminals; prostrate growth form) qualifies *Chrysanthemoides* to be separated into two species using Davis and Nixon's (1992) interpretation of phylogenetic species. Here character-based evidence is used to suggest that gene exchange does not occur between the two *Chrysanthemoides* species.

Using the autapomorphic or monophyletic species concept (Hennig 1966; de Queiroz & Donoghue 1988, 1990), one species is strongly supported. Little evidence for *C. monilifera* being a separate species is available. Bootstrap values of 0.36 were calculated for the *C. monilifera* clade. However, the evidence for *C. incana* being a distinct species is strong with a bootstrap value of 0.87. Thus on all criteria, these two taxa are distinguished at specific rank.

4.5.3. Infraspecific Classification

In most parts of South Africa, *Chrysanthemoides* is taxonomically 'difficult' because of its tendency to form different races due to its extreme vegetative plasticity (pp. 128). To arrive at a rational taxonomy, a technique must be used to determine definable units. Even though the interbreeding patterns of *Chrysanthemoides* are unknown, we can still consider geographical populations with a definable set of morphological and ecological characters as an important genetic entity.

Interpreting morphological variation over a range of geographical habitats at the infraspecific level for *Chrysanthemoides* can be tedious. Correct subdivision of formal names can often more than counterbalance the precision that is lost when names are incorrectly applied. A conservative approach will therefore be more appropriate when applying names to *Chrysanthemoides*. Names will only be applied to populations or groups of populations that are morphologically and/or eco-geographically distinct.

Under the EcSC, species are defined as a group of individuals occupying 'minimally different adaptive zones' (Van Valen 1976; Andersson 1990). In *Chrysanthemoides* the EcSC is most useful at the infraspecific level where character differences are disguised by intermediate morphologies between forms. Here, eco-geography can still be used to make taxonomic decisions since phenetic patterns may not always be conclusive. Phenetic differences at the specific level are conclusive in taxonomic decisions and an

interpretation of ecological niches is not required, even though habitats occupied by the two species may be very similar in some cases. However, at the infraspecific level, ecology may be more informative than phenetic patterns.

4.5.3.1. Subspecies

The presence of character differences in phenetic analyses which in cases may be obscure or intergrading, indicate that taxa below the species level are not sharply delimited. In *Chrysanthemoides*, a replacement sequence of morphologically varying taxa is expected for ecologically varying habitats. According to Stebbins (1950), 'subspecies are based primarily on recognizable differences, while ecotypes are distinguished by their reaction to the environment, and may or may not possess well-marked morphological differences which enable them to be recognized in the field.' Furthermore, the 'subspecies is primarily a morphological, geographical and historical concept, while the ecotype is an ecological and adaptational one.' *Chrysanthemoides* can be separated into several subspecies based on distinct morphologies and geographical distribution. Morphological intermediates in parapatric areas suggest that populations have the ability to express phenotypic plasticity in response to a changing habitat.

Intermediate morphologies at the margins of *C. incana* ssp. *subcanescens* and ssp. *incana* var. *microphylla* indicate that ssp. *subcanescens* under the BSC and CSC are not necessarily genetically and reproductively isolated. Although the subspecies are morphologically distinguishable, they are not necessarily genetically distinct which indicates subspecific status must be given. Morphologically intermediate populations have been collected from Laingsburg and Bredasdorp (*Compton* 2506; *Goldblatt* 1457; *O'Callaghan* 629; *Pillans* 12689).

Phenetic and cladistic analyses group ssp. *subcanescens* with *C.*

incana and not *C. monilifera*. On the basis of these results ssp. *subcanescens* is transferred from *C. monilifera* to *C. incana*.

The subspecies of *C. monilifera* (*canescens*, *septentrionalis*, *rotundata* and *monilifera*) recognized by Norlindh (1943) have been corroborated. All four subspecies are morphologically and eco-geographically distinct but populations may be intermediate in parapatric areas of their distribution such as ssp. *canescens* and ssp. *septentrionalis* (*Brass* 16649; *Chapman* 8037; *Muller* 2179), and ssp. *canescens* and ssp. *rotundata* (*Abbott* 2518; *Galpin* 21699; *Ward* 7182). Morphologically intermediate populations of ssp. *monilifera* and ssp. *floribunda* (form 1) have been collected at Rooiels and in the Bredasdorp district (*Burger* 1372; *Lewis* 5139; *Oliver* 5112). Intermediate morphologies suggest that *Chrysanthemoides* populations have the potential to establish themselves, but competitive exclusion may be selecting against them preventing the two subspecific populations from co-occurring.

4.5.3.2. Varieties

Stebbins (1950) describes a subspecies or geographical variety as 'a series of populations having certain morphological and physiological characteristics in common inhabiting a geographical subdivision of the range of the species, or a series of similar ecological habitats, and differing in several characteristics from typical members of other subspecies, although connected with one or more of them by a series of intergrading forms.' He suggests that using several degrees of rank may produce more confusion than order, however, the nature of the categories which are to be recognized depends on which number of ranks is most convenient and provides a clear picture of the variation in the genus.

The occurrence of local races within subspecies, with a greater number of intergrading populations, occupying tighter ecological

niches, suggest that certain subspecies may be further subdivided into varieties. Phenetic analyses identify very few character gaps, while ecological data suggest that taxa form a local distribution of a larger geographical range.

Varieties of *C. incana* ssp. *incana* are eco-geographically distinct but have large numbers of morphologically intergrading populations at the margins of their distribution. Populations are not always morphologically distinct and each variety forms a geographical subdivision of the range of the subspecies. Isolated populations of var. *hirsuta* may be found on coastal dunes in the vicinity of Cape Agulhas in the Western Cape. *C. incana* ssp. *incana* var. *incana* populations occur at Hermanus, Gans Bay and Gouritz River Mouth, up to 60 km from Cape Agulhas. *C. incana* ssp. *incana* var. *rangei* occurs from Port Nolloth in the Northern Cape, along the West Coast to Spencer Bay in Namibia. This area is continuously exposed to coastal fog and soil conditions are sandy with a low nutrient content. The area between Hondeklip Bay, Vanrhynsdorp and Port Nolloth seems to be uninhabited by *C. incana* populations. However, further south a slender leaf form, var. *gracilis* is found growing in the vicinity of Clanwilliam, Calvinia and Hondeklip Bay.

C. monilifera ssp. *pisifera*, on the basis of its eco-geography can be separated into three varieties: *C. monilifera* ssp. *pisifera* var. *borealis* is geographically isolated occurring in the Kamiesberg. However, its morphology is similar to that of var. *pisifera* forms 1 and 2, however, the leaf margins differ by being spinescent. *C. monilifera* ssp. *pisifera* var. *pisifera* forms 1 and 2 are continuously distributed from the Bredasdorp district to Kentani in the Transkei. *C. monilifera* ssp. *pisifera* var. *angustifolia* is eco-geographically isolated from all other *pisifera* populations occurring on Bokkeveld shales on the Langeberg in the Swellendam area.

4.5.3.3. Forms

Stebbins (1950) defines a form as 'a category with no genetic or evolutionary significance, since it consists of all of those individuals which possess in common some aberration from the norm of the species or subspecies.' du Rietz (1930) describes a form as 'a population of one or several biotypes occurring sporadically in a species populations (not forming distinct or regional facies of it) and differing from other biotypes of this species population in one or several characteristics.'

Ecological interpretations in conjunction with electrophoretic results and morphological analyses, suggest that *C. monilifera* ssp. *floribunda* should be separated into two forms. Genetic identity estimates indicate that *C. monilifera* ssp. *floribunda* forms 1 and 2 are very similar. Indications are that different morphologies are adaptations (due to phenotypic plasticity) to a changing environment. On the basis of these results, a putative indication is given to the separation of *C. monilifera* ssp. *pisifera* var. *pisifera* into an Eastern and Western Cape race. Forms 1 and 2 have a continuous distribution from east to west showing a cline in morphological change.

5.0. Taxonomic Treatment of *Chrysanthemoides*

***Chrysanthemoides* Tourn. ex Medik.**, Phil. Bot. 1: 159 (1789); not *Chrysanthemoides* sensu Fabricius. Type: Unknown

Eriocline Cass., Bull. Sc. Soc. Philom. Paris: 142 (1818) et Dict. Sc. Nat. 15: 191 (1819) et 30: 324 et 333 (1824). Lectotype: *Osteospermum spinosum* Willd. (1803) (non L.)

Lepisiphon Turcz., Bull. Soc. Nat. Moscou 24: 1: 180 (1851). Type: *Lepisiphon dentatus* Turcz.

Note: There seems to be confusion as to whether Medikus (Phil. Bot. 1: 159) or Fabricius (Enum. Meth. Pl.: 79) is the legitimate publisher of *Chrysanthemoides*. Indications are that Fabricius' description applies to a North American species, *Polymnia uvedalia*. Therefore *Chrysanthemoides* Fabr. should becomes a synonym of *Polymnia* L. and *Chrysanthemoides* Tourn. ex Medik. should be conserved (Nordenstam pers. comm.).

Habit a low prostrate to erect shrub or tree, 1 - 4 m tall, 1 - 7 m in diameter, plants solitary or gregarious, evergreen, single stemmed up to 0.9 m in diameter, sometimes with runners; tap root system shallow, often partially leathery; stem soft, woody, spinescent or non-spinescent, tannins present or absent.
Leaf blade succulent, leathery or thin; leaves alternate, clustered at branch terminals or arranged regularly along stem; leaf shape obovate or elliptic, 4 - 60 mm X 5 - 115 mm; young leaves pubescent, larger than older leaves; old leaves glabrous; leaf margin toothed, scolloped, spinescent or entire. *Inflorescence* corymbosely dispersed in a synflorescence (capitulescence); flower heads heterogamous, radiate, solitary or in small axillary or terminal groups, shortly peduncled, 15 - 50 mm in diameter. *Bracts* 2 - 12, in 2 - 3 rows, membranous, 2 - 5 mm long, linear, green. Inner *involucral scales* lanceolate to ovate, outer scales linear to lanceolate, smaller than inner scales. *Marginal florets* yellow, female, bearing a single petal, with three apical teeth; corolla tubes hairy, approximately 0.25 the length of marginal florets; ray styles bifurcate, stamenoids sometimes present; pappus absent. *Disc florets* female sterile, corolla tubes funnel shaped, glandular at base, hairy, sterile;

florets 5-lobed with elongated stamens, anthers yellow or purple-black when mature. *Drupes* arranged around the edge of the receptacle, fleshy, orange-red to purple-black when mature, globose, 4.0 - 8.5 mm in diameter, globose, subglobose or ovate (drupe length/drupe breadth ratio 1.1 - 1.9); sometimes ridged. *Cytology* 2n = 20 (Norlindh 1963).

5.1. Key to the Species, Subspecies and Varieties of *Chrysanthemoides*

1. Branch apices pungent, spinescent .....2.
1. Branch apices not pungent, non-spinescent .....7.

2. Plants glabrous or with sparse pubescence; leaf margins spinescent or entire; through the Cape Karoo from Clanwilliam, Mossel Bay and east to Port Elizabeth .....*C. incana* ssp *subcanescens*
2. Plants pubescent; leaf margins toothed or entire; along the coast and inland from Gouritz River Mouth to Spencer Bay .....3.

3. Mature leaves more than 19 mm long; glabrous or pubescent or with apparently peeling pubescence; along the coastline from the Gouritz River Mouth to Saldanha .....*C. incana* ssp. *incana* var. *incana*
3. Mature leaves less than 19 mm long; plants covered with a persistent hairy pubescence; along the west coast from Saldanha to Spencer Bay in Namibia, populations occurring inland at Calvinia, Clanwilliam and at Cape Agulhas.....4.

4. Leaves narrowly elliptic; from Hondeklip Bay to Calvinia and Clanwilliam .....*C. incana* ssp. *incana* var. *gracilis*
4. Leaves obovate; along the west coast from Saldanha to Spencer Bay in Namibia, and at Cape Agulhas .....5.

5. Plants forming dome-shaped shrubs; from Clanwilliam to Saldanha .....*C. incana* ssp. *incana* var. *microphylla*
5. Plants mat-forming; from Spencer Bay in Namibia to Kleinsee and Vanrhynsdorp, and at Cape Agulhas.....6.

6. Leaves clustered at branch terminals; along the west coast from Kleinsee to Spencer Bay in Namibia, populations occurring at Vanrhynsdorp .....*C. incana* ssp. *incana* var. *rangei*
6. Leaves evenly arranged along stem; at Cape Agulhas .....*C. incana* ssp. *incana* var. *hirsuta*

7. Mature leaves leathery; plants coastal and inland .....8.
7. Leaves not leathery; plants found inland .....11.

8. Leaf margins largely toothed; drupes whitish when immature turning purple-black with maturity; from Clanwilliam east to Grahamstown, always found inland
.....*C. monilifera* ssp. *pisifera* var. *pisifera* (form 2)
8. Leaf margins serrated or entire; drupes not necessarily whitish when immature but purple-black at maturity; in the Kamiesberg area and along the coast from Saldanha to Mozambique
.....9.

9. Leaves elliptic; leaf margins spinescent; from the Northern Cape, particularly the Kamiesberg
.....*C. monilifera* ssp. *pisifera* var. *borealis*
9. Leaves oblanceolate to widely obovate; leaf margins entire or scolloped; from the Cape Peninsula along the coast to Mozambique
.....10.

10. Leaves broadly obovate, leaf ratio (length/breadth) approximately 1; from Saldanha to Humansdorp along the coastline
.....*C. monilifera* ssp. *floribunda* (form 1)
10. Leaves narrowly obovate, leaf ratio (length/breadth) approximately 1.5 - 2.0; may occur inland but generally a coastal subspecies from Knysna to Inharrime in Mozambique
.....*C. monilifera* ssp. *rotundata*

11. Involucral scales linear to narrowly ovate; from the Western Cape, Eastern Cape, along the Drakensberg mountain range and further north to Tanzania .....12.
11. Involucral scales broadly ovate; from Swellendam to Mossel Bay, forest margins from Mossel bay to the Keurbooms River Mouth, from Grahamstown to Kentani in the Eastern Cape
.....15.

12. Leaf margins entire or minutely spinescent; from the Transvaal (Lydenberg/Pilgrims Rest area), north to Tanzania at

fairly high altitudes
.....*C. monilifera* ssp. *septentrionalis*
12. Leaf margins dentate; found in the Western Cape, Eastern Cape and the Transvaal, Natal, and Lesotho Drakensberg .....13.

13. Branches and receptacles pubescent; leaves glabrous or sparsely pubescent; from Natal, the Transvaal and Lesotho Drakensberg.....*C. monilifera* ssp. *canescens*
13. Branches glabrous, receptacles sparsely covered; leaves glabrous; from the Eastern and Western Cape .....14.

14. Drupes ovate; from the Eastern Cape
.....*C. monilifera* ssp. *pisifera* var. *pisifera* (form 1)
14. Drupes globose/sub-globose; from the Western Cape (Hottentots-Holland; Piketberg; Table Mountain area)
.....*C. monilifera* ssp. *monilifera*

15. Leaf margin dentate; Leaf ratio (length/breadth) usually small (1.56); from Grahamstown to Kentani in the Eastern Cape
.....*C. monilifera* ssp. *pisifera* var. *pisifera* (form 1)
15. Leaf margins scolloped; Leaf ratio (length/breadth) usually large (2.14); from Swellendam to Mossel Bay and forest margins from George to the Keurbooms River.....16.

16. Involucral scales broadly ovate and pubescent at their margins; young leaves and capitula clustered at branch apices; occurring within forest margins from George to the Keurbooms River .....*C. monilifera* ssp. *floribunda* (form 2)
16. Involucral scales ovate, no or little pubescence on plant; leaves distributed evenly along the stems; along mountain slopes from the Swellendam/Caledon area to Mossel Bay
.....*C. monilifera* spp. *pisifera* var. *angustifolia*

5.2. Numerical Key (Figs. 14 - 18).

5.2.1. Methods

This is a multi-entry key, designed to use all available information for determining specimens. It should be particularly useful for establishing the affinities of intermediate specimens. To use this coded key, a choice should be made at each letter. Numerical morphology codes are then listed in their respective order followed by the choice of distribution found under the Distribution Code heading. The sequence of numerals should be compared to those in Table 26 to identify your specimen.

5.2.2. Morphological Codes

A) Plants spinescent.....1.
   non-spinescent.....2.
B) Leaf shape oblanceolate/obovate.....1.
   broadly or narrowly elliptic.....2.
C) Leaf margin scolloped.....1.
   toothed/serrate.....2.
   spinescent.....3.
   entire.....4.
D) Leaves leathery.....1.
   notleathery.....2.
E) Leaves arranged in terminal clusters.....1.
   arranged evenly along stems.....2.
F) Leaves covered with a hairy pubescence.....1.
   partly covered or naked.....2.
G) Drupes globose/sub-globose.....1.
   elongated or ovoid.....2.

5.2.3. Distribution Codes (H)

From Pilgrims Rest, north to Zimbabwe (Masvingo; Mutare), Malawi (Blantyre northwards), southern Kenya and north east Tanzania; at high altitudes .....1.

Along the Transvaal, Swaziland, Orange Free State and Natal Drakensberg to Pietermaritzburg; found in the Orange Free State (Ficksburg; Senekal); at high altitudes .....2.

From Inharrime in Mozambique along the coastline to the Port Elizabeth vicinity in the Eastern Cape .....3.

Along the coastline from Cape Town to the Knysna district; further inland in the Bredasdorp area .....4.

From Saldanha to Clanwilliam, in the Wuppertal district and isolated populations in the Bredasdorp area .....5.

Found on the Cape Agulhas coastline .....6.

From northern Clanwilliam, to Calvinia and Hondeklip Bay.....7.

Along the coastline from Spencer Bay in Namibia to Kleinsee, but may occur inland at Vanrhynsdorp .....8.

Isolated to the Kamiesberg and northward to Vioolsdrift.....9.

Found in the dry interior, from Knysna to Umtata in the Transkei; concentrated in the Grahamstown area along dry mountain slopes and along river beds .....10.

From Bredasdorp in the Western Cape, north to Wuppertal and East to Grahamstown; plants usually isolated along the dry slopes or dry river beds .....11.

Along the coastline from Saldanha to the Gouritz River Mouth .....12.

Forest margins from George to the Keurbooms River Mouth .....13.

From as far north as Piketberg, and east to Worcester and Hermanus.....14.

Interior of the Cape Province along dry water courses; from Clanwilliam towards the south coast at Humansdorp (Swartkops river) and Port Elizabeth district .....15.

On gentle mountain slopes at higher altitudes in the Caledon/Swellendam areas to Mossel Bay .....16.

Table 26: Morphological and distribution codes for *Chrysanthemoides* taxa (see Table 1b for abbreviation of taxon names).

Morphology Code							**Distribution Code**	**Taxon**
A	B	C	D	E	F	G	H	
2	1/2	2	2	2	2	1	14	*C.mon.mon.*
2	1	1	1	1/2	2	2	4	*C.mon.flo.*F1
2	2	1/3	2	2	2	2	13	*C.mon.flo.*F2
2	2	2	2	2	2	2	10	*C.mon.pis.*F1
2	2	2	1	2	2	2	11	*C.mon.pis.*F2
2	2	3	1	2	2	2	9	*C.mon.pis.bor.*
2	2	1/3	2	2	2	2	16	*C.mon.pis.ang.*
2	2	2	2	2	1	2	2	*C.mon.can.*
2	2/4	3	2	2	2	2	1	*C.mon.sep.*
2	1	1/4	1	2	2	2	3	*C.mon.rot.*
1	1	3	1	2	2	2	12	*C.inc.inc.*
1	1	3	1	2	1/2	2	5	*C.inc.mic.*
1	1	3	1	1	1	2	8	*C.inc.ran.*
1	1	4	1	2	1	2	6	*C.inc.hir.*
1	2	3/4	1	2	1	2	7	*C.inc.grac.*
1	2	3	2	2	2	2	15	*C.inc.sub.*

Chrysanthemoides monilifera (L.) T. Norl., Stud. Calend.: 374 (1943); Adamson & Salter, Fl. Cap. Pen.: 825 (1950); Gibson, Wild Flow. Natal: 114 fig. 3 (1975); Dyer, Gen. S. Af. Flow. Pl. 1: 715 (1975); Gray, Misc. Notes Aust. Pl. 2. *Chrysanthemoides*: 2 (1976); Hilliard, Comp. Natal: 526 (1977); Bond & Goldblatt, Pl. Cap. Fl.: 160 (1984); Palgrave, Trees of Southern Africa.: 913 (1984); Breitenbach, Nat. List Indig. Trees: 189 (1986); Weiss, J. Aust. Ins. Agric. Sci. 52: 127 (1986); Moll, Trees Natal: 199 (1992).

Osteospermum moniliferum L., Sp. Pl: 923 (1753); Syst. Nat. 10: 1234 (1759) et. Sp. Pl. 2: 1308 (1763); Mill., Fig. Pl. Descr. Gard. Dict.: 757 tab. 194 fig. 1 (1760); Mant., Pl: 480 (1771); Bergius, Descr. Pl. Cap.: 330 (1767); Mill., Gartn. -Lex. 3: 360 (1776); Aiton, Hort. Kew. 3: 275 (1789); Lam., Encycl.: tab. 714 (1797-1819); Savigny in Lam., Encycl. 4: 660 (1797); Thunb., Prodr. Pl. Cap.: 167 (1800); Schult. Fl. Cap.: 715 (1823); DC., Prodr. Regn. Veg. 6: 460 (1837).

Type: *Chrysanthemoides africanum, populi albae foliis.* Dill., Hort. Elth.: 80 tab. 68 fig. 79 (1732) selected by Norlindh (1943)

Erect shrub or small tree, 1 - 4 m tall, 1 - 6 m in diameter, root system shallow, woody, pubescence varying in thickness, non-spinescent. *Leaves* elliptic to obovate, 0.4 - 0.6 mm thick, 7 - 40 mm X 10 - 150 mm, glabrous or pubescent, tannins present or absent, margins toothed entire spinescent or scolloped, petiole 6 - 60 mm long. Inner and outer *involucral scales* linear to ovate, glabrous or pubescent, 3 - 10 mm long. *Inflorescence* a capitula 1 - 10 flowers, heterogamous, shortly peduncled, 15 - 50 mm in diameter. *Marginal florets* yellow, female, fertile, 13 - 20 mm long, corolla tubes cylindric, petals with 3 apical teeth, stamens may be present, styles bifurcate. *Disc florets* male, purple-black. *Drupes* globose, ovoid or elongated, 3.5 - 7.0 mm long, drupe length/drupe breadth ratio 1.1 - 3.75, drupe ridging present or absent.

Usually flowering from April to December.
Drupes present from June to March.

Chrysanthemoides monilifera* (L.) T. Norl. ssp. *monilifera

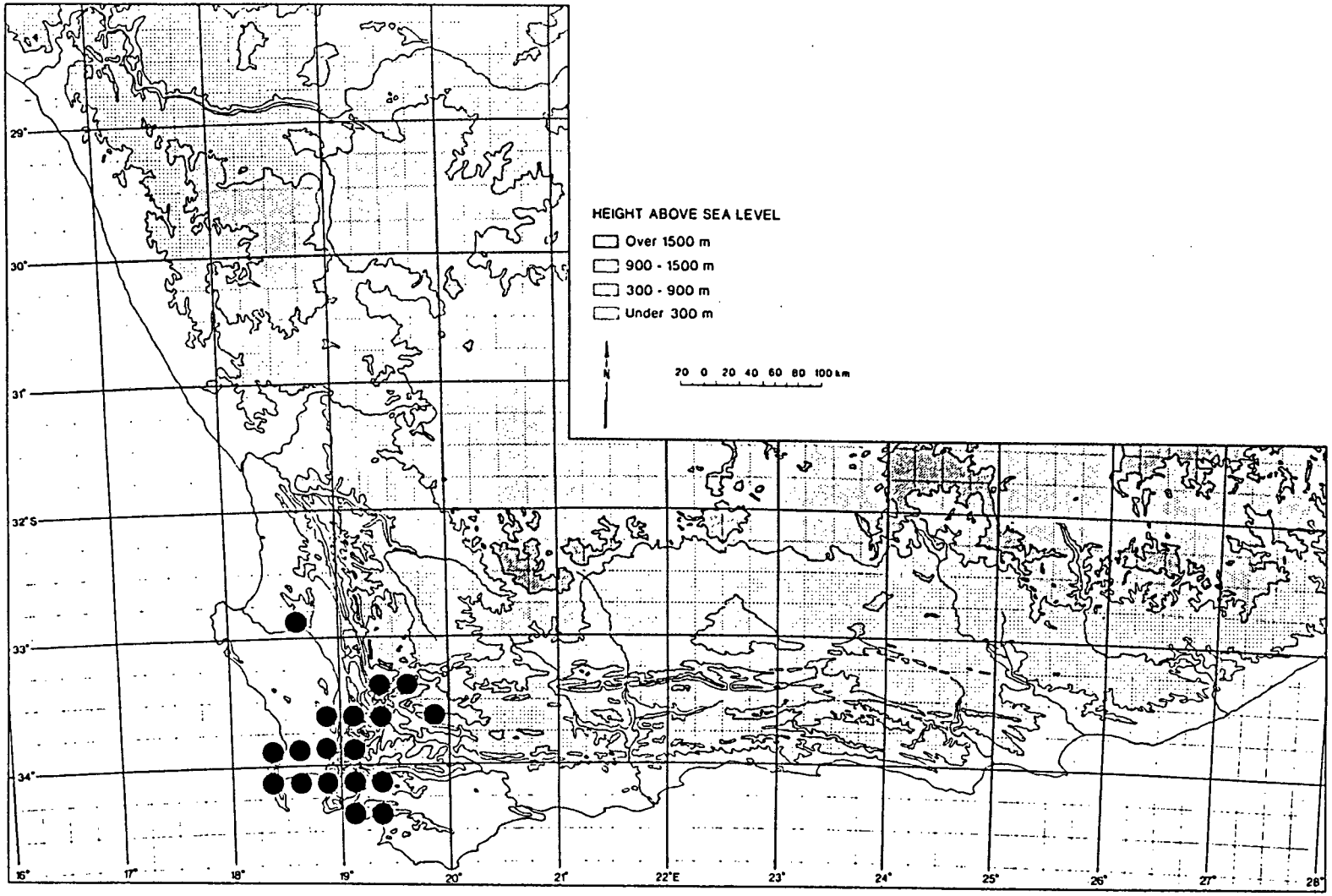
Note: A description of the subspecies is given by Dillenius (Hort. Eltham.: tab. 68 fig. 79 (1732)). Norlindh (1943) includes in his description of *C. monilifera* (L.) T. Norl. ssp. *monilifera*, both globose and sub-globose fruited forms. However, for his lectotype he has chosen an illustration of a plant which looks like *C. monilifera* (L.) T. Norl. ssp. *floribunda* R.C. Griffioen which has ovoid fruits. The lectotypification has to be followed since botanical nomenclature rules state 'the author who first designated a lectotype or a neotype must be followed, but this choice is superseded if it can be shown that it is in serious conflict with the protologue, and another element is available which is not in conflict with the protologue' (Greuter 1988, Article 8.1c). Linnaeus (Sp. Pl.: 923 (1753)) was the first botanist to describe the species calling it *Osteospermum moniliferum* adding the phrase *Osteospermum foliis ovalibus serratis*. This description states nothing about fruit shape.

Shrub, 1.5 m in height X 2.0 m in diameter. *Stems* tanniferous, pubescence absent. *Leaves* elliptic or slightly obovate, 0.4 - 0.6 mm thick, 7 - 30 mm X 20 - 50 mm, glabrous, tannins absent, pubescence absent, petiole 6 - 20 mm long, margins toothed. Inner *involucral scales* lanceolate, outer involucral scales linear, slightly pubescent, 4 - 7 mm long. *Drupe* fleshy, orange-red when mature, globose to sub-globose, 5 - 8 mm in diameter, drupe length/drupe breadth ratio 1.1 - 1.2, drupe ridging absent.

Ecology and Distribution: The subspecies is found in disturbed sites on the margins of climax vegetation which includes genera such as *Protea, Leucadendron, Podalyria, Virgilia,* and *Widdringtonia* (Acocks 1988). Populations are found growing on mountain slopes as far north as Piketberg and east to Worcester and Hermanus (Map 1). Although the subspecies may be confused with *C. monilifera* ssp. *pisifera* (L.) T. Norl. var. *pisifera*, it has globose rather than ovoid fruits, and their distributions do not overlap. Plants grow in soils such as Table Mountain

sandstone, limestone ridges and loams. Populations frequent coastal fynbos rather than coastal renosterveld (Acocks vegetation type 46 & 47).

Selected specimens: Western Cape, Stellenbosch, Stellenbosch Dam, 3318 DD, *Smit* 2 (STE); Western Cape, Riversdale, Takkiesfontein, 1978, *Hugo* 1235 (STE); Western Cape, Hermanus, 1942, *Barker* 2014 (NGB); Western Cape, Piketberg Mountain, 1949, *Martin* 243 (NGB); Western Cape, Paarl Mountain Reserve, Christmas Camp, 3318 DB, 1988, *Martin* 57 (STE).



Map 1: Distribution of *C. monilifera* ssp. *monilifera*.

***Chrysanthemoides monilifera* (L.) T. Norl. ssp. *floribunda* R.C. Griffioen.**, ssp. nov.; folia alterna petiolata; lamina subcoriacea obovata-oblanceolata 7 - 40 mm lata 10 - 60 mm longa margine leviter cartilaginea serrata; pubescenceum in ramis terminalia persistens; putamina obovoidea (non globosa). Type: Western Cape, Caledon, Bettys Bay, 3418 BD, 1982, *Burman* 838 (NBG!).

C. monilifera sensu Burman, S.A. Wild Flower Guide 5: 192 (1985); Palmer & Pitman, Trees of Southern Africa: 2163 - 2165 vol. 3 (1972).

Forming a small to large, erect bush, 1 - 3.5 m in height X 1 - 6 m in diameter. *Stems* tanniferous, pubescence absent from young tissues. *Leaves* elliptic to broadly elliptic, 6 - 60 mm X 10 - 150 mm, leaf thickness varies considerably, 0.6 - 2.5 mm, glabrous, tannins absent, young tissue pubescent, clustered at branch terminals, margins spinescent or scolloped, petiole 6 - 10 mm long. Inner and outer *involucral scales* narrowly lanceolate to ovate, glabrous or pubescent, 4 - 7 mm long. *Drupes* fleshy, purple-black when mature, ovoid, drupe length/drupe breadth ratio 1.4 - 2.3. Drupe ridging present or absent.

Two forms may be recognized in the subspecies:

Form 1: *Leaves* obovate (6 - 40 mm X 10 - 50 mm), leaf margins spinescent or scolloped, lamina leathery or fleshy. Young leaves and flowers clusters at branch terminals, covered with a loose pubescence. *Drupes* ovoid, 4 - 6 mm long, drupe ridging present.

Ecology and Distribution: Populations of form 1 grow along coastal sand dunes and further inland where they intergrade with *C. monilifera* ssp. *pisifera* var. *pisifera*. Plants are distributed from Langebaan on the west coast to the Knysna district (Map 2). Populations are found growing on limestone outcrops, sand dunes or secondary areas alongside roads. The subspecific epithet *'floribunda'* is derived from the spectacular sight of this subspecies in full flower.

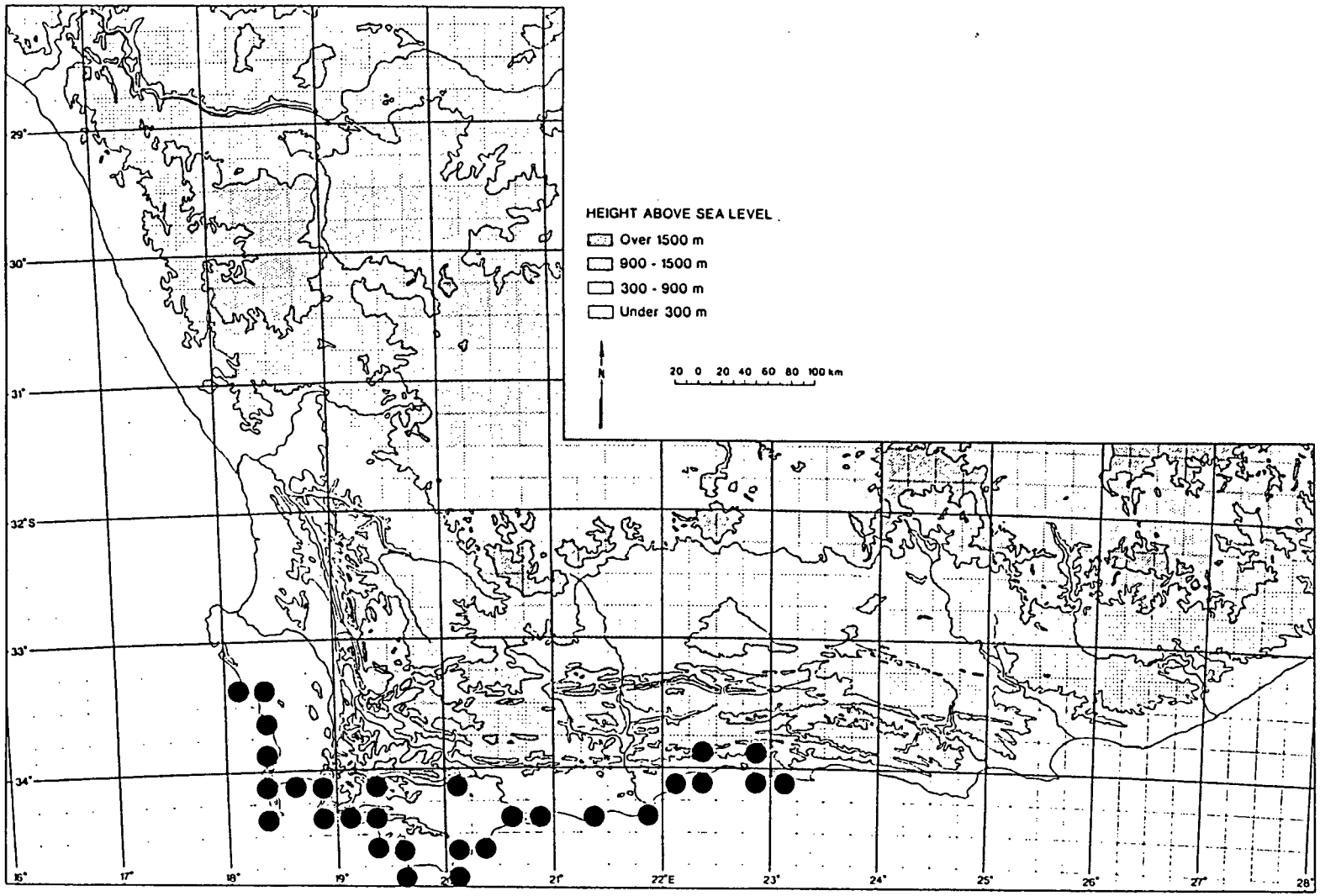
Selected specimens: Western Cape, Caledon, Bettys Bay, Harold Porter Botanic Gardens, 3418 BD, 1973, *Ebersohn* 323 (NBG); Western Cape, Witsand, 1943, *Wasserfall* 242 (NGB); Western Cape,

Gans Bay, 1946, *Compton* 18158 (NGB); Western Cape, between Vissers Hok and Mamre Rd, 1946, *Leighton* 1761 (BOL).

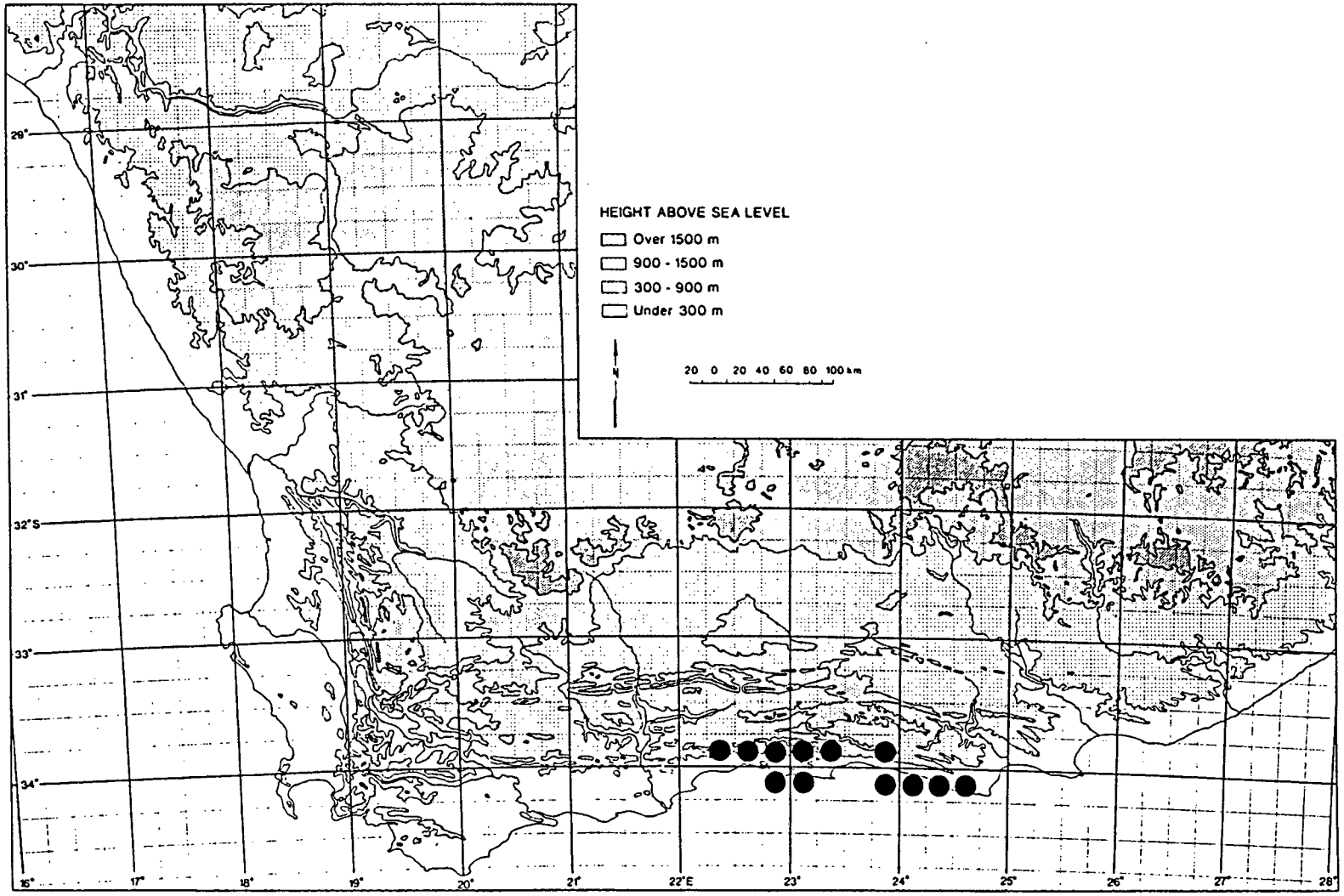
Form 2: *Leaves* narrowly elliptic to broadly elliptic, leaf size very variable, 15 - 60 mm X 40 - 100 mm, leaf margins scolloped, leaf lamina thin. *Involucral scales* broadly ovate, covered with a loose pubescence. *Marginal florets* 20 - 30 mm. *Drupes* ovoid, 4 - 6 mm long, drupe ridging absent.

Ecology and Distribution: Coastal populations of form 1 have spread into forest margins in the Knysna and George areas (Map 3). Limited light conditions cause plants to increase their leaf size, grow taller and leaves become more slender. Soils are shallow, fertile and have a high humus content.

Selected specimens: Eastern Cape, Knysna, Mouth of Keurbooms River, 1938, *Gillett* 4549 (BOL); Eastern Cape, George, Montagu Pass, 1931, *Thorne* 51637 (BOL); Eastern Cape, Humansdorp, Blaauwkrantz, 1949, *Morris* 425 (NGB); Eastern Cape, Wilderness, George, 1923, *Levyns* 784 (BOL).



Map 2: Distribution of *C. monilifera* ssp. *floribunda* (form 1).



Map 3: Distribution of *C. monilifera* ssp. *floribunda* (form 2).

***Chrysanthemoides monilifera* (L.) T. Norl. ssp. *pisifera* (L.) T.** Norl., Stud. Calend.: 383 (1943).

Osteospermum pisiferum L., Syst. Nat. 10: 1234 (1759); Sp. Pl. 2: 1308 (1763); Mill., Fig. Pl. Gard. Dict.: tab. 194 fig. 1 (1757); Mant. Pl.: 480 (1771); Bergius, Descr. Pl. Cap.: 330 (1767); Mill., Gartn. -Lex. 3: 360 (1776); Aiton., Hort. Kew. 3: 275 (1789); Savigny in Lam. Encycl. 4: 660 (1797); Thunb., Prodr. Pl. Cap.: 167 (1800); Schult., Fl. Cap.: 715 (1823), (sub nom. *O. piliferum*, err. typ.); Lodd., Bot. Cab.: 470 tab. 470 (1821); DC., Prodr. Regn. Veg. 6: 460 (1837); Loud., Arboret. et Fruticet. Brit. 2: 1072 fig. 848-850 (1838).

Osteospermum ciliatum Burm. (sensu *Osteospermum pisiferum* L.) Berg., Descr. Pl. Cap.: 330 (1767); DC., Prodr. Regn. Veg. 6: 465 (1837).

Osteospermum moniliferum var. *pisiferum* (L.) Harv. et Sond., Fl. Cap. 3: 436 (1865).

Chrysanthemoides pisiformis (*Osteospermum pisiforme* L., err. typ.) Medik., Phil. Bot. 1: 159 (1789).

Type: *Osteospermum foliis lanceolatis acute dentatis, caule fruticoso*. Mill., Gard. Dict.: tab. 194 fig. 1 (1757).

Notes: A good account of the nomenclature of the subspecies is given by Norlindh (1943). One of Miller's engraved plates (Gard. Dict.: 129 fig. 1 (1760)) illustrates this subspecies in colour. Linnaeus (Syst. Nat. 10: 1234 (1759)) describes both Miller's (Gard. Dict. (1768)) illustration and a different plant (*Osteospermum ciliatum*) illustrated by Burman (Prodr. Fl. Cap.: 171 tab. 169 fig. 2 (1738)) under the species *Osteospermum pisiferum*. Bergius discovered this mistake by Linnaeus and called the later specimen described by Burman, *Osteospermum ciliatum*. De Candolle (Prodr. Regn. Veg. 6: 465 (1837)) later cited Burman's description of the taxon, describing both *Osteospermum pisiferum* and *Osteospermum ciliatum*.

Stems tanniferous, pubescence absent. *Leaves* narrowly elliptic to elliptic, glabrous, margins scolloped or toothed, tannins absent, petiole 8 - 20 mm long. Inner *involucral scales* narrowly ovate or ovate, outer involucral scales lanceolate, slightly pubescent, 3 - 10 mm long. *Drupes* fleshy, purple-black when mature, obovoid, 3.0 - 5.5 mm, drupe ridging present.

C. monilifera (L.) T. Norl. ssp. _pisifera_ (L.) T. Norl. var. _pisifera_

Two forms may be recognized in the variety:

Form 1: Plant taller than the next three varieties forming large bushes or a small tree, 2.0 m in diameter X 2.5 m high. *Leaves* 15 - 30 mm X 25 - 45 mm, elliptic, glabrous, leaf margins 3 - 5 dentate. *Involucral scales* lanceolate, 3 - 7 mm long, with a sparse pubescence. *Drupes* ovate, 4 - 6 mm long.

Ecology and Geographical Variation: Distribution ranging from Grahamstown to the Transkei (Map 4). Plants occur in false fynbos or fynbos on sand on gentle slopes, and not necessarily disturbed ground. Soils are shallow, but fertile. The area receives 150 - 400 mm of rain per annum at an altitude of 1000 - 1500 m above sea level.

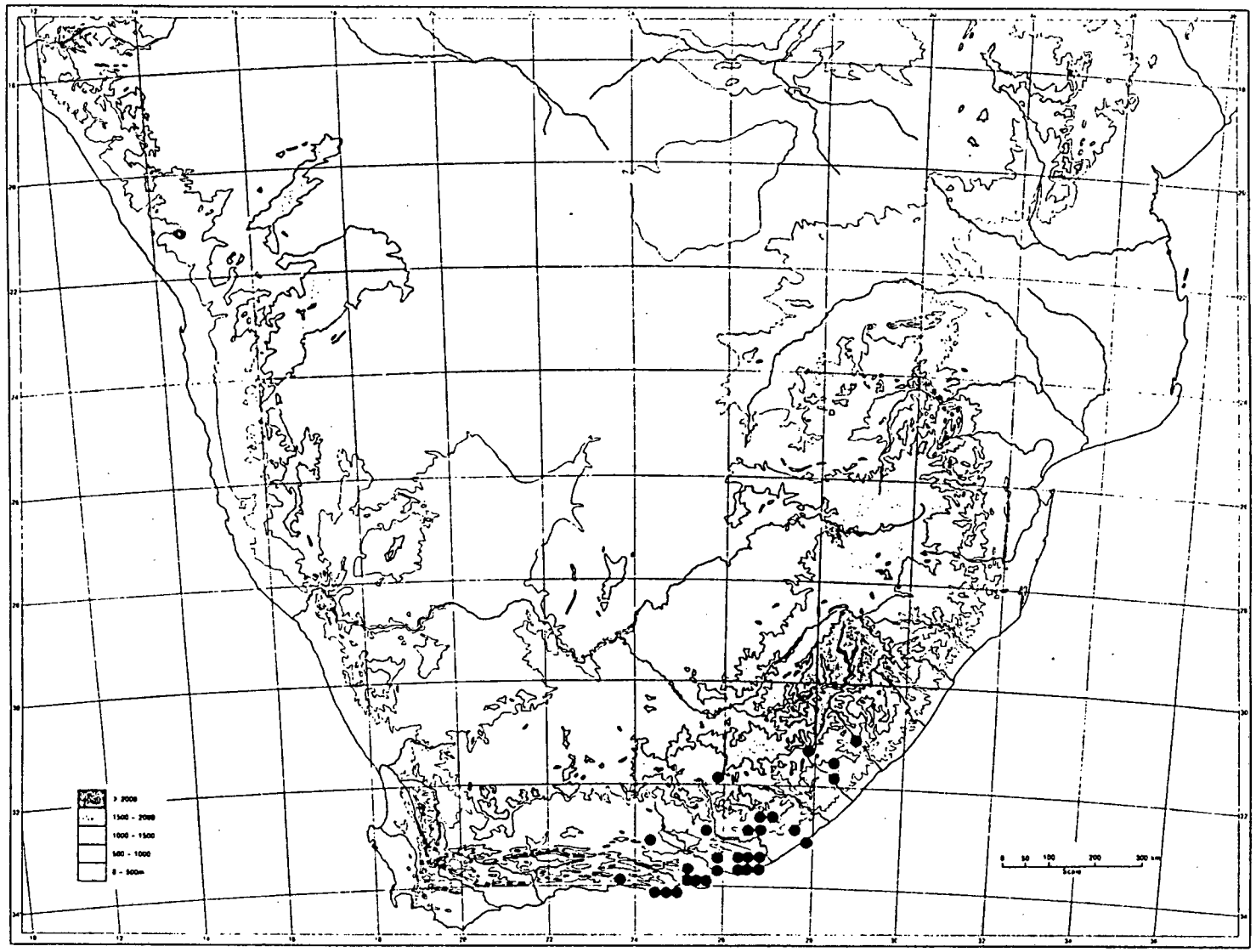
Selected specimens: Eastern Cape, Port Elizabeth, Van Stadens Flower Reserve, 1961 *Dahlstrand* 1970 (NGB); Eastern Cape, Grahamstown, Howiesons Poort, 1931, *Rennie* 85 (NGB); Eastern Cape, Port Elizabeth, Mangold Park, Baakens River, 1974, *Olivier* 1097 (NGB); Eastern Cape, Grahamstown, Albany, 1759 *Lussem* 36 (NGB); Eastern Cape, Uniondale, slopes of Kouga Mountain, Braam River, 1949, *Esterhuysen* 16337 (NGB).

Form 2: A small shrub to 1.5 m high. *Leaf* size variable, 5 - 30 mm X 8 - 45 mm, elliptic, leathery, deeply dentate, 2 - 4 teeth per leaf margin, glabrous. *Involucral scales* lanceolate, 4 - 10 mm, little or no pubescence. *Drupes* ovoid, white when immature turning purple-black with maturity, 4 - 6 mm long.

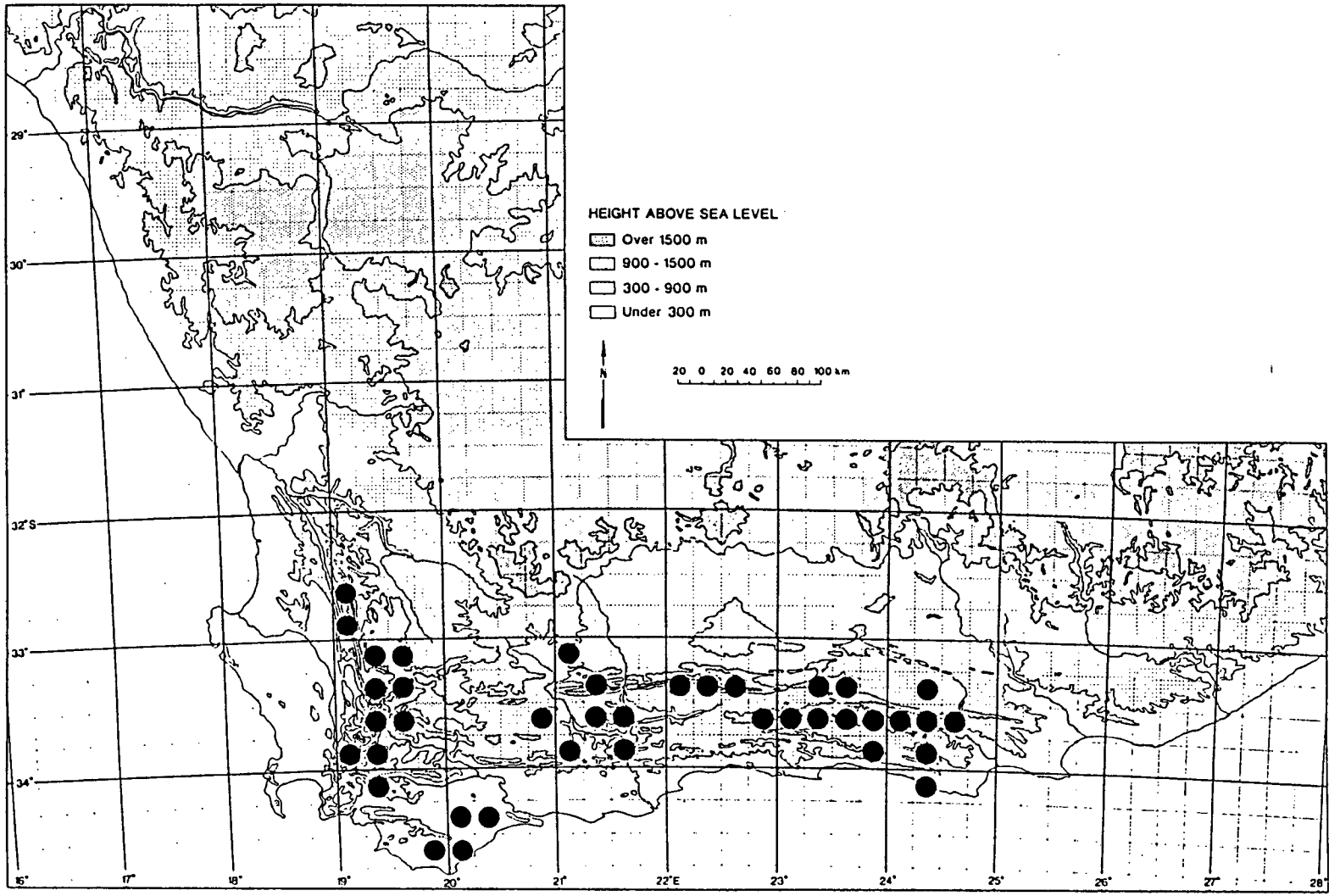
Ecology and Distribution: Distribution ranging from Bredasdorp to Uniondale and Humansdorp (Map 5). Specimens collected from Karoopoort close to Ceres have large leaves (25 mm X 40 mm) which are conspicuously dentate (*Compton* 22930). Plants may occur in disturbed ground where soils are derived from sandstone and conglomerates which may be rocky or clayey. Plants occur in mountain renosterbosveld (Acocks vegetation type 43) and rich soils in river gulleys. Leaf size increases towards the Western

Cape, however, in the Eastern Cape, leaves are very small (7 - 15 mm X 10 - 20 mm). Plants occur at altitudes ranging from 20 - 900 m above sea level, which receives 25 - 300 mm of rain per annum.

Selected specimens: Eastern Cape, Willowmore, Joubertina/Kouga, 3323 BB, 1975, *Geldenhuys* 346 (STE); Western Cape, Bredasdorp, Potberg, 1954, *Marguire* 2594 (NGB); Western Cape, Ladismith, Rooiberg, 3321 AD, 1969, *Rycroft* 3044 (NGB); Western Cape, Karoo Poort, Ceres, 1951, *Compton* 22930 (NBG).



Map 4: Distribution of *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1).



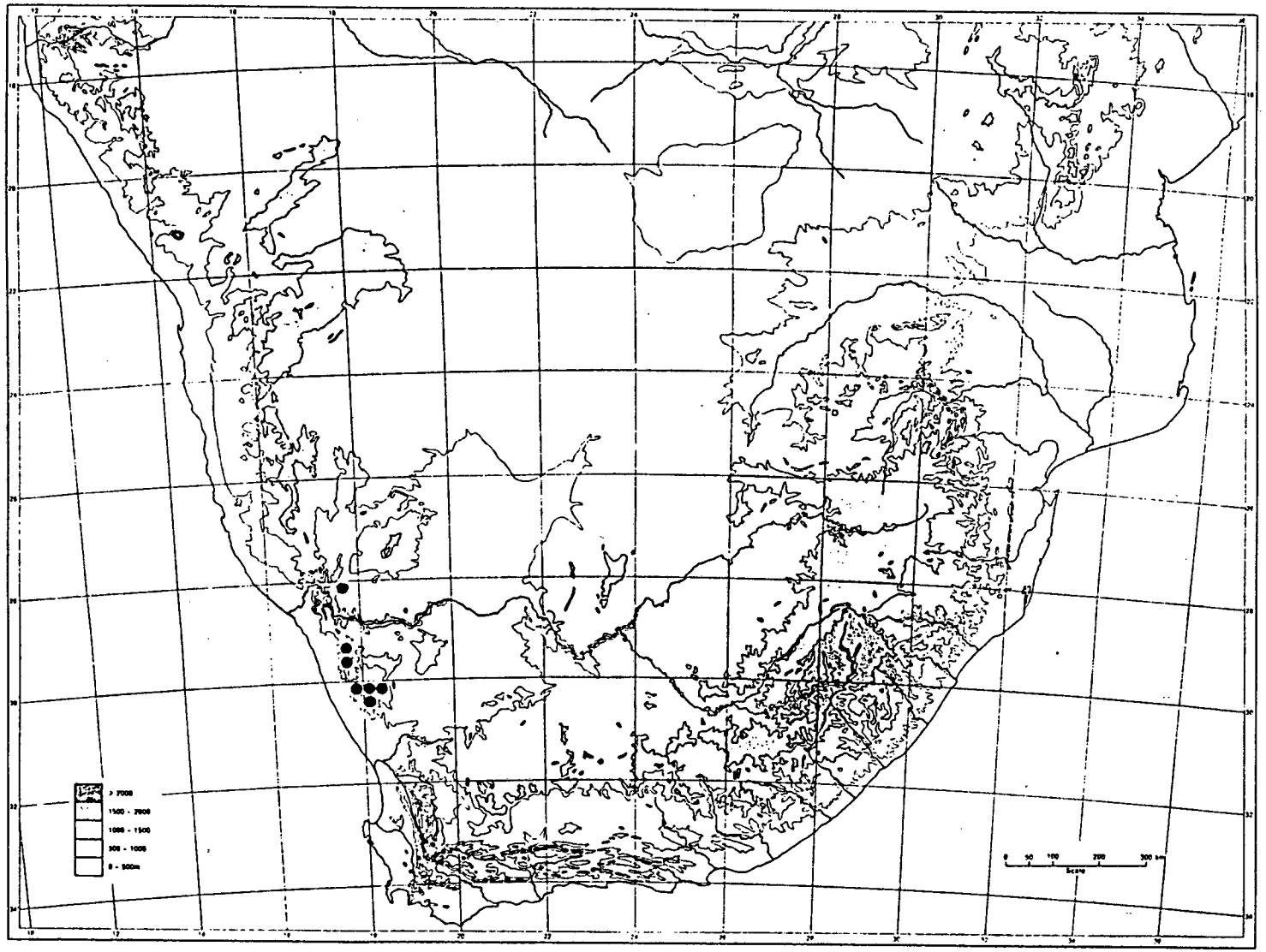
Map 5: Distribution of *C. monilifera* ssp. *pisifera* var. *pisifera* (form 2).

C. monilifera **(L.) T. Norl. ssp.** *pisifera* **(L.) T. Norl. var.** *borealis* **R.C. Griffioen**, var. nov.; folia parva modo 30 - 50 mm longa elliptica margina 2 - 4 serrata.
Type: Cape Province, Vioolsberg, Ploegberg, 2817 CA, 1989, *Viviers* 2094 (NGB!).

Stems without tannins, pubescence absent. *Leaves* partially leathery, narrowly elliptic, 10 - 20 mm X 25 - 45 mm, glabrous, leaf margins spinescent. Inner and outer *involucral scales* lanceolate, 4 - 9 mm long, glabrous. *Marginal florets* 12 - 22 mm long. *Drupes* ovate.

Ecology and Distribution: I have not seen any live populations (herbarium material seen) of this variety, therefore little comment is offered on their growth habit. Plants are found growing in the Kamiesberg and the surrounding Namaqualand (Map 6). The variety is geographically isolated from all other populations of *Chrysanthemoides*, and forms the most northerly range for the species in the Northern Cape. Plants may be found growing at altitudes from 1000 - 1500 m above sea level, in a rainfall area of 150 - 200 mm per annum, occurring in soils derived from dwyka tillite, quartzite, clayey shales and sandstone.

Selected specimens: Northern Cape, Kamiesberg, Wilgehout Ravine, 1929, *Pearson* 6798 (BOL); Northern Cape, Kamiesberg, Sneeukop, 1928, *Hutchinson* 876 (BOL); Northern Cape, Kamiesbergpas, Anenasplaas, 1983, *van Wyk* 6421 (PRE); Northern Cape, Namaqualand, Spektakel Hill, 1951, *Compton* 22809 (BOL).



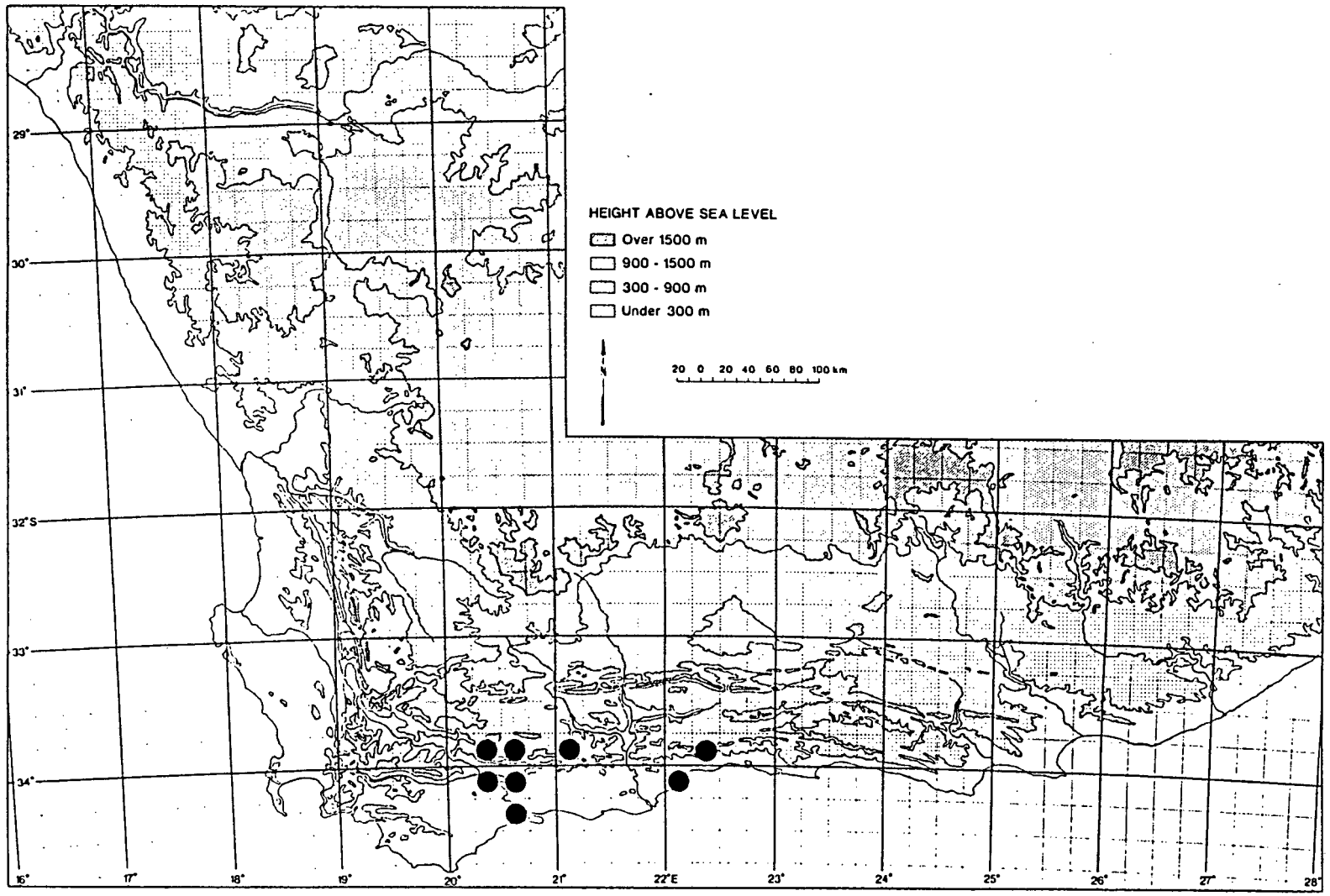
Map 6: Distribution of *C. monilifera* ssp. *pisifera* var. *borealis*.

***C. monilifera* (L.) T. Norl. ssp. *pisifera* (L.) T. Norl. var. *angustifolia* R.C. Griffioen**, var. nov.; folia parva modo 10 - 25 mm lata 35 - 60 mm longa anguste elliptica putamina obovoidea (non globosa). Type: Western Cape, Langeberg, Boesmansbos Wilderness Area, 3320 DD, *McDonald* 1588 (STE!).

Erect bush, 1 - 2 m tall X 1 - 2 m in diameter. *Leaves* narrowly elliptic, 10 - 25 mm X 35 - 60 mm, glabrous, leaf margins scolloped. Inner and outer *involucral scales* partly pubescent, ovate. *Drupes* ovoid, purple-black when mature.

Ecology and Distribution: This variety occurs in Coastal Renosterbosveld and False Macchia (Acocks vegetation type 46;70) in disturbed areas (road sides; alongside cultivated land), as well as natural areas. Plants restricted to moist Bokkeveld shales, sandy acid flats and clayey soil of the Overberg in the vicinity of Swellendam and Caledon (Map 7). Due to extensive wheat cultivation, the natural vegetation is scarce or in a rather poor condition and this variety is now rather restricted in its distribution.

Selected specimens: Western Cape, Montagu, Swellendam State Forest, 1978, *Haynes* 1466 (STE); Western Cape, Zuurbraak to Barrydale, Tradouw Pass, 3320 DC, 1968, *Marsh* 882 (STE); Western Cape, Swellendam, Heidelberg, 1949, *Morris* 278 (NGB); Western Cape, Caledon, Vogelgat Kloof, 3419 AD, 1979, *Williams* 2840 (NGB).



Map 7: Distribution of *C. monilifera* ssp. *pisifera* var. *angustifolia*.

***Chrysanthemoides monilifera* (L.) T. Norl. ssp. *canescens* (DC.)** T. Norl., Stud. Calend.: 395 (1943); Hilliard, Comp. Natal: 528, (1977); Van Wyk & Malan, Field Guide to Wild Flowers of the Witwatersrand and Pretoria Region.: 100 (1988).

Osteospermum pisiferum var. *canescens* DC., Prodr. Regn. Veg. 6: 460 (1837); Drège., Flora. 2. Beigabe: 52 (1843);

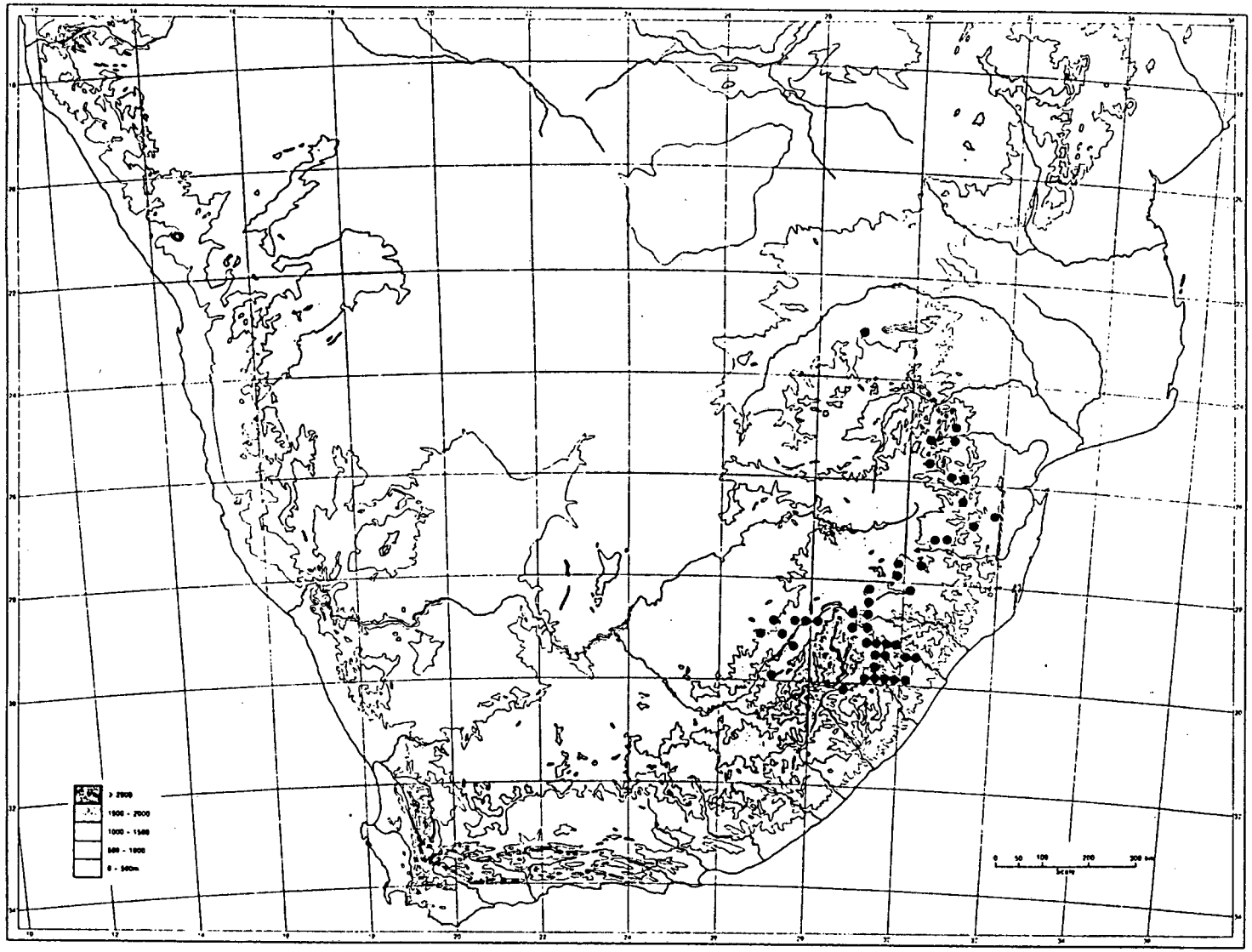
Type: Witbergen, *Drège* 6129 (G) selected by Norlindh (1943)

Stems pubescent, tannins absent. *Leaves* elliptic to broadly elliptic, 0.3 - 0.5 mm thick, 10 - 35 mm X 25 - 55 mm, pubescent, tannins absent, leaf margins toothed, petiole 5 - 20 mm long. Inner *involucral scales* slightly ovate, outer involucral scales lanceolate, pubescent, 5 - 7 mm long. *Drupes* obovoid, purple-black when mature, 2 - 6 mm long, drupe length/drupe breadth ratio 2, drupe ridging present.

Ecology and Distribution: At low altitudes, stems and leaves lose their pubescence. Leaf size varies considerably (10 - 30 X 15 - 50 mm), however, the leaf margin is always strongly dentate. Plants occur in *Protea* vegetation and grasslands along the Drakensberg mountain range through Natal, Transvaal and Lesotho, and the Suikerbosrand (Map 8) where dominant grasses belong to *Themeda*, *Hyparrhenia* and *Cymbopogon*. Plants occur in poor soils derived from sandstone and granite. The Drakensberg receives an annual rainfall of 350 - 600 mm, at an altitude of 1000 - 2500 m above sea level.

Plants flower throughout the year.

Selected specimens: Natal, Royal Natal National Park, Mahai streamside, 1992, *Vos & Gromley* 370 (NU); Natal, Cathedral Peak Forest Reserve, 1982, *Manning* 243 (NU); Natal, Escourt, Gladstone Nose, 1968, *Wright* 473 (NU); Natal, Impendle, Loteni Nature Reserve, 1978, *Phelan* 183 (NU).



Map 8: Distribution of *C. monilifera* ssp. *canescens*.

***Chrysanthemoides monilifera* (L.) T. Norl. ssp. *septentrionalis* T. Norl.**, Stud. Calend.: 396 (1943); Agnew. Upland Kenyan Wild Flowers: 486 (1974).

Osteospermum moniliferum forma foliis subintegris Engl., Hochgeb. -fl. Trop. Afr. in Abh. Akad. d. Wiss. Berlin: 447 (1892).

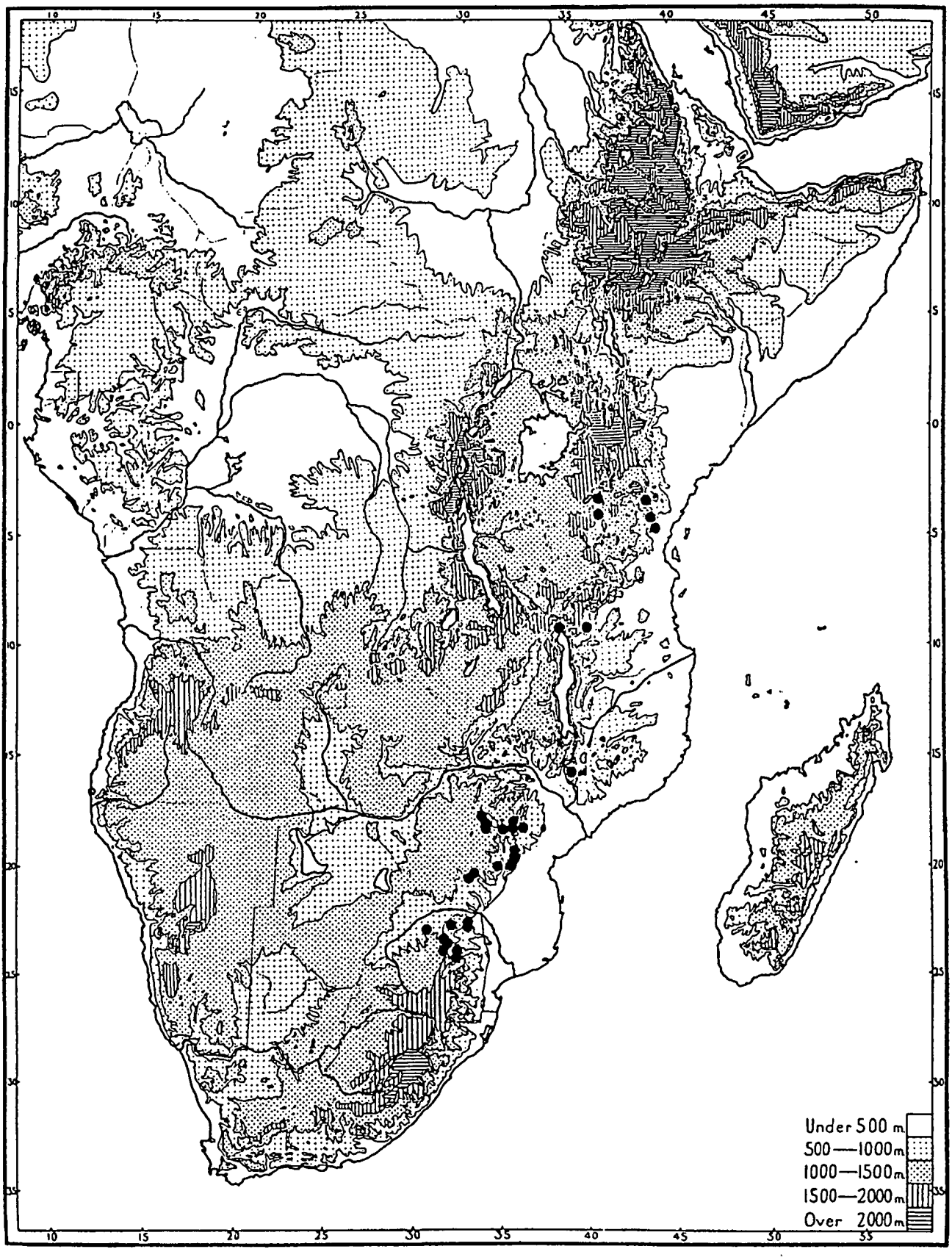
Ostespermum moniliferum L.: Engl., Pfl. -welt Ost-Afr. C.: 419 (1895).

Type: Rusapi, Makoni District, 1931, *Norlindh et Weimarck* 4166 (LD).

Erect shrub or bush. *Stems* without tannins, pubescent. *Leaves* elliptic to narrowly elliptic, 0.3 - 0.5 mm thick, 7 - 25 mm X 10 - 40 mm, pubescent or glabrous, tannins absent, leaf margins entire or spinescent, petiole 4 - 12 mm long. *Inflorescence* 19 - 25 mm in diameter. Inner *involucral scales* ovate, outer involucral scales broadly lanceolate, slightly pubescent, 3 - 7 mm long. *Drupes* purple-black when mature, obovoid, drupe length/drupe breadth ratio 2, 3 - 6 mm long, drupe ridging present.

Ecology and Distribution: A tropical montane subspecies occurring at altitudes of 1500 - 2400 m above sea level in Zimbabwe, Malawi, southern Kenya and Tanzania (Map 9). Plants occur in montane grassland and woodlands. At altitudes of 2000 m above sea level, leaves and stems are pubescent and plants resemble *C. monilifera* ssp. *canescens* (Luchenya Plateau, Mulanje Mountain, Malawi (*Hilliard & Burtt* 4163); Inyanga, Zimbabwe (*Brass* 16649)). This subspecies is usually found growing on disturbed sites such as fire breaks, termite mounds and pastures. Plants favour soils derived from dolerite with a shallow topsoil (300 - 400 mm) in a rainfall area of 350 - 600 mm per annum.

Selected specimens: Umtali, Manica, Odzani River Valley, 1914, *Teague* 219 (BOL); Harare, Sans Souri Rd, 1976, *Daillecourt* 33 (NU); Inyanga, Demera, 1974, *Nicholas* 211 (NU); Malawi, Biniwini and Dedza, 1967, *Hilliard & Burtt* 4163 (NU).



Map 9: Distribution of *C. monilifera* ssp. *septentrionalis*.

***Chrysanthemoides monilifera* (L.) T. Norl. ssp. *rotundata* (DC.)** T. Norl., Stud. Calend.: 391 (1943); Gibson, Wild Flow. Natal: 114 fig. 3 (1975); Gray, Miscel. Notes Aust. Pl. 2. *Chrysanthemoides*: 4 (1976); Hilliard, Comp. Natal: 526 (1977); Weiss, J. Aust. Ins. Agric. Sci. 52: 128 (1986); Pooley, Trees Natal, Zululand, Transkei: 493 (1993);

Osteospermum rotundatum DC., Prodr. Regn. Veg. 6: 461 (1837). *Osteospermum moniliferum* var. *rotundatum* (DC.) Harv. et Sonder, Fl. Cap. 3: 436 (1865).

Osteospermum moniliferum sensu Wood & Evans, Natal. Pl. 1: 46 tab. 55. (1899).

Osteospermum macrocarpum DC., Prodr. Regn. Veg. 6: 461 (1837). Type: Umkomaas River, Natal, Drège 5051 (G) selected by Norlindh (1943).

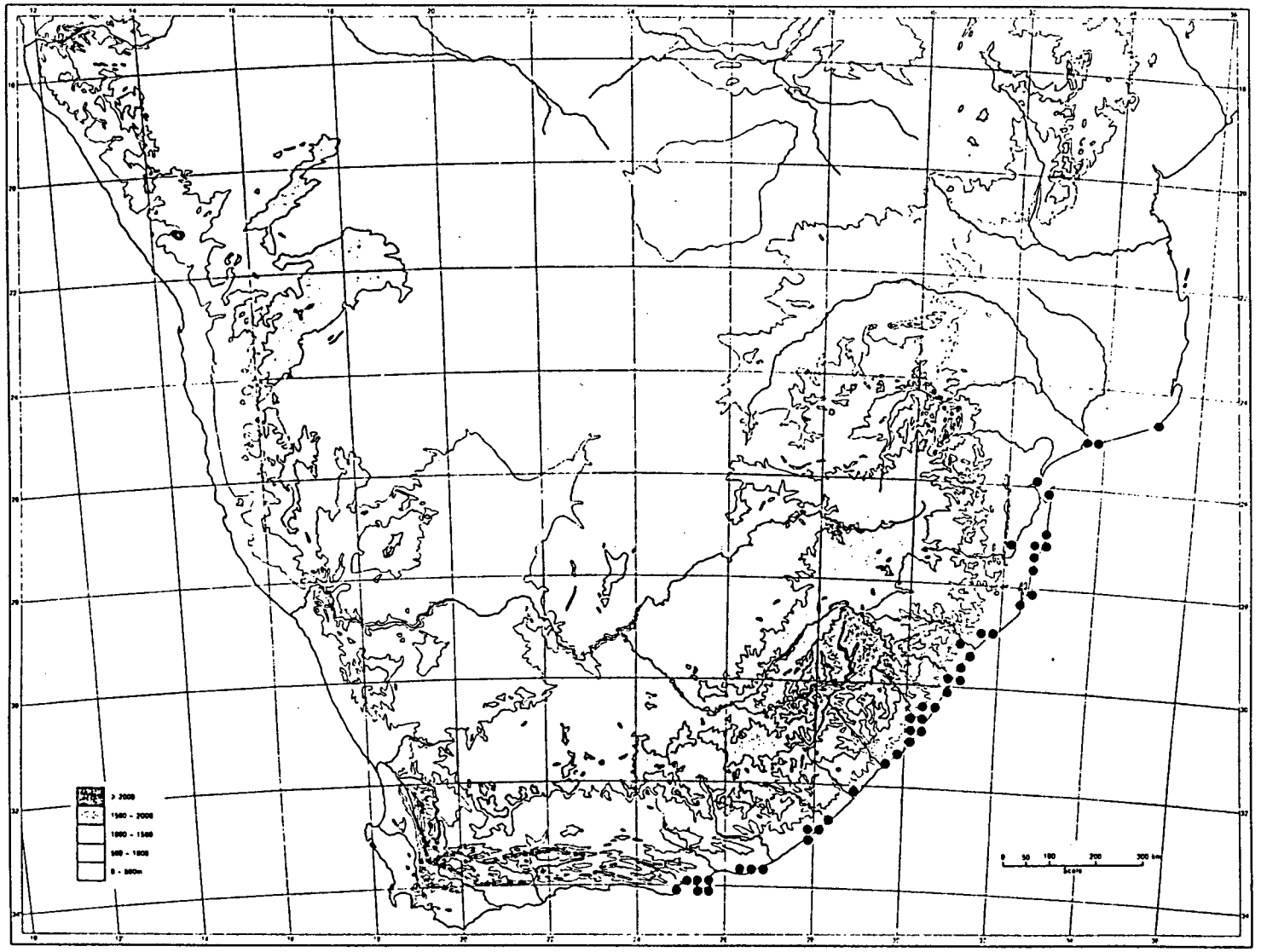
Notes: *Osteospermum macrocarpum* in the synonym must be clarified since *O. junceum* and *C. monilifera* ssp. *rotundata* are included in this species. Synonymy depends on the typification of *C. monilifera* ssp. *rotundata* which in this case is Drège 5051 (G) selected by Norlindh (1943).

Scrambling shrub or small tree. *Stem* tanniferous, glabrous. *Leaves* obovate to broadly obovate, 0.2 - 1.5 mm thick, 15 - 60 mm X 30 - 70 mm, mature leaves glabrous, tanniferous, young leaves clustered at branch apices, pubescent, margins entire or scolloped, petiole 6 - 25 mm long. Inner *involucral scales* lanceolate or narrowly ovate, outer involucral scales lanceolate, slightly pubescent or glabrous, 2.5 - 5.0 mm long. *Drupes* fleshy, purple-black when mature, obovoid, drupe length/drupe breadth ratio 1.67 - 2, 3 - 6 mm long, drupe ridging present.

Plants flower throughout the year.

Ecology and Distribution: Distributed from Port Elizabeth to Inharrime in Mozambique (Map 10). Isolated populations occur inland at Umtamnvuna Nature Reserve, Port Edward (*Abbott* 1662). Plants grow on weakly developed, sandy soils where lime may be present or absent, but tolerate a wide range of edaphic factors. The distribution of the subspecies appears to be restricted by water availability, not occurring further south-west that Port Elizabeth. The subspecies occurs at altitudes of 0 - 150 m above sea level with a rainfall of 250 - 500 mm per annum.

Selected specimens: Natal, Port Shepstone, Umtetweni Station, 1950, *Barker* 6156 (NGB); Eastern Cape, Port Elizabeth, 1930, *Fries, Norlindh & Weimarck* 169 (BOL); Natal, Pinetown, Eventon, 1963, *Hilliard* 1505 (NU); Eastern Cape, Port Edward, 1959, *Simpson* 150 (NU).



Map 10: Distribution of *C. monilifera* ssp. *rotundata*.

***Chrysanthemoides incana* (Burm. f.) T. Norl.** Stud. Calend.: 399 (1943); Adamson & Salter, Fl. Cap. Pen.: 825 (1950); Dyer, Gen. S. Af. Flow. Pl. 1: 715 (1975); Van Jaarsveld, Veld & Fl. 66: 119 (1980); Kidd, Cap. Pen.: 136 fig. 10 (1983); Palgrave, Trees Sthn. Afr.: 913 (1984); Bond & Goldblatt. Pl. Cap. Fl.: 160 (1984); Breitenbach, Nat. List Ind. Trees: 189 (1986).

Osteospermum incanum (Burm. f.), Prodr. Fl. Cap.: 29 (1768).

Osteospermum spinosum Jacq., Hort. Schoenbr. 3: 66 (1798); Willd., Sp. Pl. 3: 2365 (1804).

Eriocline obovate Cass., Dict. Sc. Nat. 15: 191 (1819).

Osteospermum spinescence DC., Prodr. Regn. Veg. 6: 459 (1837); Drège, Fl. 2 Beigade: 108 (1843).

Osteospermum moniliferum var. *lanosum* (DC.), Prodr. Regn. Veg. 6: 460 (1837); Drège Fl. 2: 47 (1843); Harv. & Sond., Fl. Cap. 3: 446 (1865);

Osteospermum lanosum (DC.), Compt. & Pill., Trans. Roy. Soc. S. Afr. 19: 33: 324 (1931).

Type: In Herb. Burman (G).

Prostrate shrub or small erect bush, 0.2 - 2.5 m tall, 1 - 7 m in diameter, root system penetrating soft sandy soils, tanniferous, stem pubescent, spinescent. *Leaves* narrowly elliptic, narrowly obovate to broadly obovate, 0.2 - 1.0 mm thick, 5 - 30 mm X 5 - 35 mm, alternate, pubescent or partially pubescent, margins toothed or entire, tannins absent, petiole 5 - 15 mm long. *Inflorescence* with 1 - 10 capitula, heterogamous, shortly peduncled, 15 - 30 mm in diameter. *Marginal florets* 8 - 13 mm long, corolla tubes cylindric. *Disc florets* purple-black. *Involucral scales* ovate, small, slightly pubescent or naked, 2 - 5 mm long. *Drupes* 5 - 7 mm long, purple-black when mature, ovoid, drupe length/drupe breadth ratio 1.65 - 2.33, drupe ridging absent.

Flowering throughout the year.

Drupes usually present from July to January.

Chrysanthemoides incana* (Burm. f.) T. Norl. ssp. *incana

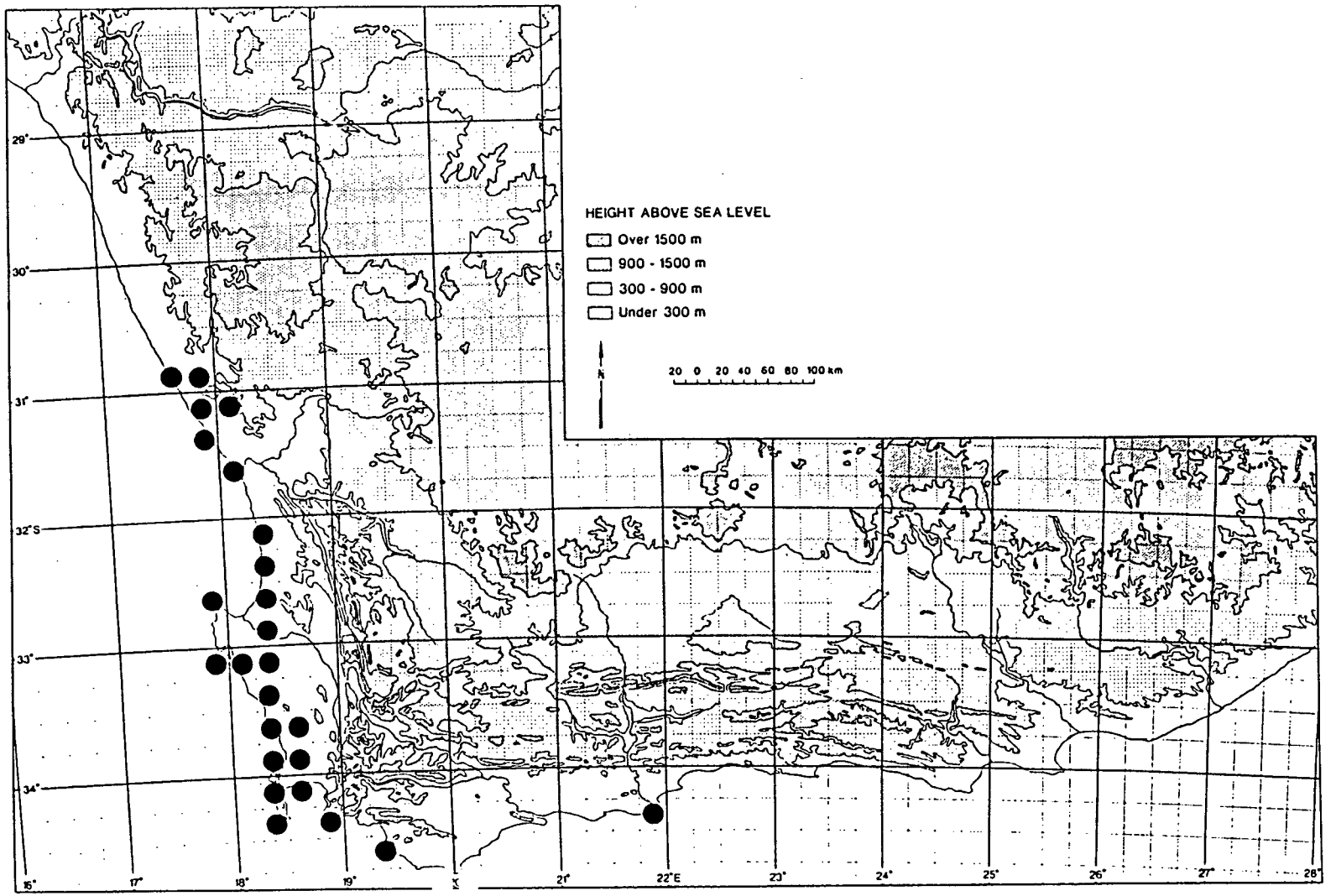
Bushes prostrate or forming a small shrub, 0.2 - 1.5 m tall, 1 - 7 m in diameter, young tissues always pubescent. *Stems* with peeling pubescence, plants spinescent or with pungent branch terminals, tannins absent. *Leaves* elliptic, narrowly obovate to broadly obovate, 5 - 35 mm X 7 - 40 mm, alternate, pubescent or glabrous, tannins absent, margins toothed or entire, petioles 2 - 15 mm long. *Involucral scales* covered with pubescence. *Drupes* ovoid.

Chrysanthemoides incana* (Burm. f.) T. Norl. ssp. *incana* var. *incana

Bushes prostrate or forming a small shrub, 0.4 - 1.0 m tall, 1 - 4 m in diameter. *Stems* with peeling pubescence, plants spinescent. *Leaves* broadly obovate, 10 - 35 mm X 20 - 40 mm, alternate, pubescence peeling or glabrous, margins toothed. *Involucral scales* ovate, 2 - 5 mm long, pubescent. *Drupes* ovoid, 5 - 7 mm long, drupe ridging absent.

Ecology and Distribution: Distributed along the coastline from Gouritz river mouth (*Callaghan* 308), to Saldanha, and further north with a patchy distribution up to Vredendal (Map 11). Leaf size decreases for plants found further north. Isolated populations have been collected from the Cederberg Mountains (*van Breda* 4574). The subspecies is often found growing in association with *C. monilifera* ssp. *monilifera* where the latter has a stunted growth form. Plants have been collected at Gans Bay (*Parsons* 133) and Simonstown. The area receives a rainfall of 100 - 500 mm per annum. The variety grows in well drained, sandy soils, sandy soil over lime and granite derived soils.

Selected specimens: Western Cape, Milnerton, Killarney Hotel, 1994, *Barker* 5369 (NGB); Western Cape, Hopefield, 1946, *Compton* 18927 (NGB); Western Cape, Saldanha, Danger Bay, 1946, *Leighton* 1735 (BOL); Western Cape, Paarden Eiland, Klein Zoar Vlei, 1979, *Linder* 2164 (BOL).



Map 11: ● Distribution of *C. incana* ssp. *incana* var. *incana*.
■ Distribution of *C. incana* ssp. *incana* var. *hirsuta*.

Chrysanthemoides incana **(Burm. f.) T. Norl. ssp.** *incana* **var.** *microphylla* **R.C. Griffioen**, var. nov.; ramuli spinescentes folia obovata - oblanceolata 5 - 10 mm lata 10 - 20 mm longa margine serrata. Type: Western Cape, Clanwilliam, Notier Reserve, 1946, *Leipoldt* 4240 (BOL!).

Bushes prostrate or forming a small shrub, 0.4 - 2 m tall, 1 - 3 m in diameter. *Stems* long, pubescent, plants spinescent. *Leaves* obovate, 5 - 15 mm X 10 - 20 mm, alternate, pubescent, margins toothed. *Involucral scales* ovate, 2 - 5 mm long, pubescent. *Drupes* ovoid, drupe ridging absent. Plants flowering early in the year from February to September.

Ecology and Distribution: Some plants collected from Paternoster have black leaves (5 - 10 mm X 10 - 20 mm), due to fungus growth. The variety is distributed from Velddrift and further north to Clanwilliam (Map 12). Isolated populations have been collected in Bredasdorp (*O'Callaghan* 629) and Laingsburg (*Compton* 2506) district. The variety may be found growing with *Pelargonium* at altitudes of 0 - 500 m above sea level, the area described receives an annual rainfall of 150 - 200 mm per annum. Plants are restricted to clayey soils and littoral dunes in Namaqualand Broken Vegetation and Strandveld (Acocks vegetation types 33 & 34).

Selected specimens: Western Cape, Clanwilliam, Veldrift, St Helena Bay, 1969, *Thompson* 806 (STE); Western Cape, Vredendal, Draaihoek Farm, 1986, *Hilton-Taylor* 1160 (STE); Western Cape, Melkbosch turning, 1946 *Compton* 18109 (NBG); Western Cape, Ceres, rocky ridge above Winkelhaaks River, Bokkeveld, Sneeukop, 1946, *Esterhuysen* 12689 (NGB); Western Cape, Darling Flower Reserve, Malmesbury, 1956, *Rycroft* 1913 (NGB).

***Chrysanthemoides incana* (Burm. f.) T. Norl. ssp. *incana* var. *rangei* (R. Muschl.) R.C. Griffioen**, com. nov. et stat. nov.;
Osteospermum rangei Muschl.: Engl., Pfl. -welt. Afr. 1: 2: 515 (1910); Muschl. in Engl., Bot. Jahrb. 66: 117 (1911). Type: Namibia, Pomona, 1929, *Dinter* 6354 (BOL!).

Prostrate shrub at 0.5 m high. *Stems* producing leaves at their terminals, pubescent. *Leaves* obovate, 4 - 5 mm X 5 - 15 mm, leathery, pubescent, clustered at branch terminals, leaf margin toothed. *Involucral scales* pubescent. *Marginal florets* 5 - 10 mm. *Drupes* ovoid, 4.5 - 7.0 mm long.

Ecology and Distribution: I have not seen plants in their natural habitat therefore little comment is offered on their growth habit. Distributed along the coast from Spencer Bay in Namibia to Kleinsee in the north-western Cape (Map 12). Populations occur in the Van Rhynsdorp district on the Langeberg summit at altitudes of 1100 m above sea level (*Marloth* 5381). Plants appear to derive most of their moisture from the regular sea fogs along this coastline as rainfall is limited and erratic. The clustering of leaves at branch terminals could aid with the intake of water.

Selected specimens: Namibia, Luderitzbucht, 1922, *Dinter* 3830 (BOL); Namibia, Luderitzbucht, 1907, *Range* 498 (BOL); Northern Cape, Port Nolloth, Groenplaat, 1978, *Le Roux & Ramsey* 164 (STE); Northern Cape, Little Namaqualand, S of Holgat River, *Pillans* 5190 (BOL); Northern Cape, State land, Port Nolloth, Groenplaat, Muisvlakte, 1975, *Le Roux* 164 (PRE); Northern Cape, Little Namaqualand, Holgat River, *Pillans* 5190 (BOL).

***Chrysanthemoides incana* (Burm. f.) T. Norl. ssp. *incana* var. *hirsuta* R.C. Griffioen**, var. nov.; frutex prostratus 0.3 - 1.0 m altus dense ramosus ramuli spinescentes folia obovata - oblanceolata 7 - 25 mm lata 10 - 30 mm longa margine serrata indumentum in ramis involucro foliisque perdiu persistens. Type: Eastern Cape, Bredasdorp, Cape Agulhas, coastal bush, *Levyns* 11538 (BOL!).

Prostrate bush at 0.5 m high. *Stems* pubescent. *Leaves* broadly obovate, 7 - 25 mm X 10 - 30 mm, leathery, pubescent, leaf margins entire or dentate. *Involucral scales* pubescent, 3 - 5 mm long.

Ecology and Distribution: Found at Cape Agulhas on coastal sand dunes (Map 11) at an altitude of 0 - 20 m above sea level. The Cape Agulhas area receives an annual rainfall of 150 - 200 mm, the area is characterized by sands or sandy limestones. Plants have stems, leaves and involucral scales pubescent, their growth form prostrate but spinescent.

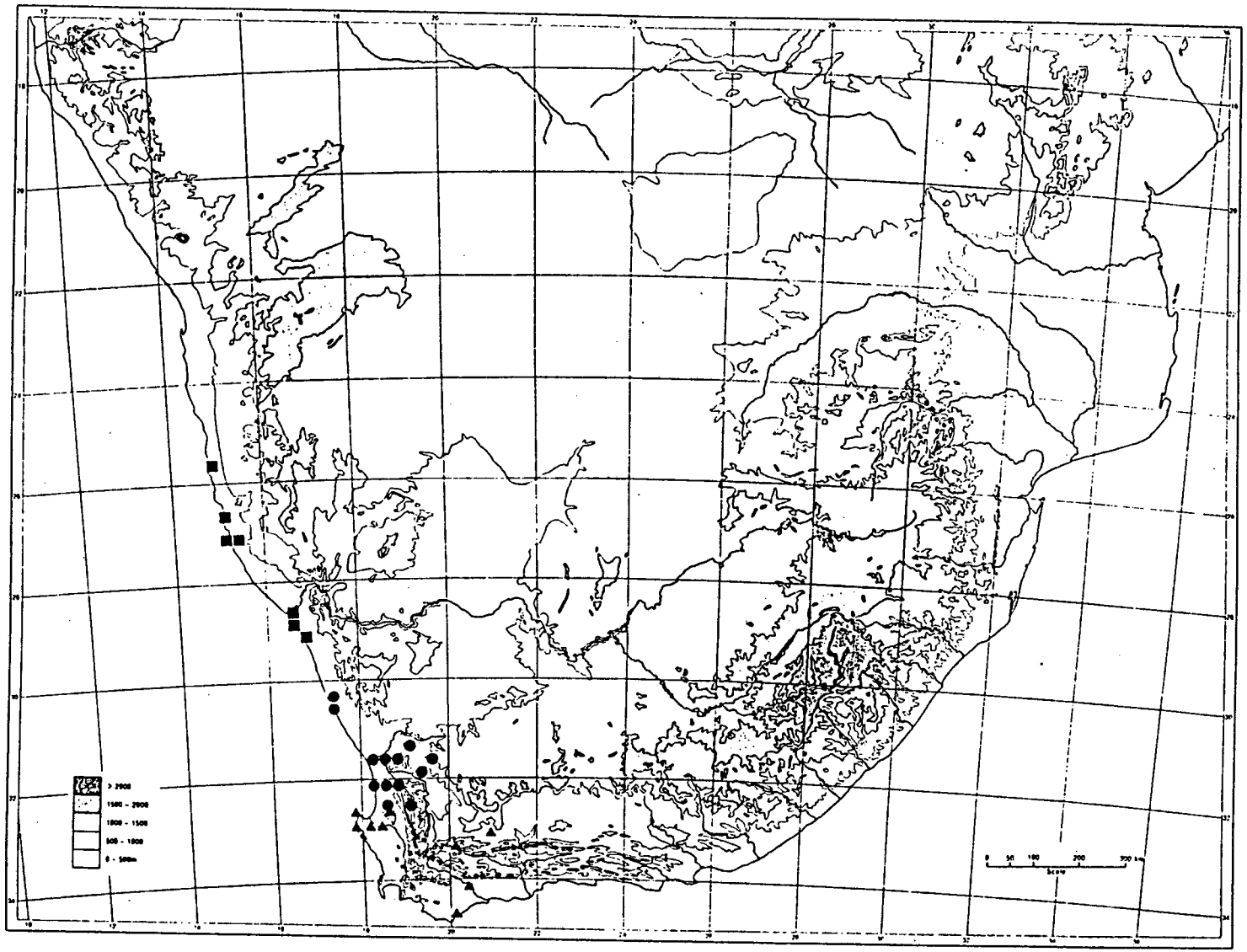
Selected specimens: Western Cape, Bredasdorp, Cape Agulhas, 1934, *Salter* 4823 (BOL); 1935, *Pillans* 8139 (BOL); 1979, *Taylor* 10165 (STE); 1966, *Levyns* 11538 (BOL).

***Chrysanthemoides incana* (Burm. f.) T. Norl. ssp. *incana* var. *gracilis* R.C. Griffioen**, var. nov.; ramuli spinescentes folia parva modo 3 - 12 mm lata 10 - 30 mm longa anguste elliptica margine integro. Type: Cape Province, Clanwilliam, Nardouw, 1947, *Compton* 20046 (NBG!).

Plants forming a low spreading, much branched bush. *Stems* slender, pubescent. *Leaves* narrowly elliptic, 3 - 12 mm X 10 - 30 mm, pubescent, partially leathery. *Marginal florets* 5 - 10 mm. *Drupes* ovoid, 3.5 - 7.0 mm long.

Ecology and Distribution: Distributed from Clanwilliam to Calvinia and westwards to Hondeklip Bay (Map 12). The plants receive an annual rainfall of 50 - 175 mm, at altitudes of 20 - 700 m above sea level. Populations are similar to *C. incana* ssp. *subcanescens*, but may be distinguished by pubescent stems, leaves and involucral scales.

Selected specimens: Northen Cape, Calvinia, Botterkloof Pass, 1950, *Barker* 6504 (NBG); Northern Cape, Hondeklip Bay and Swart Lentjies River, 1924, *Pillans* 18109 (BOL); Cape Province, Stormsvlei Kloof, 1940, *Esterhuysen* 48411 (BOL); Western Cape, Clanwilliam, 1925, *Levyns* 1238 (BOL); Northern Cape, Hondeklip Bay, 1981, *Hugo* 2877 (STE).



Map 12:

▲ Distribution of *C. incana* ssp. *incana* var. *microfolia*.
■ Distribution of *C. incana* ssp. *incana* var. *rangei*.
● Distribution of *C. incana* ssp. *incana* var. *gracilis*.

Chrysanthemoides incana (Burm. f.) T. Norl. ssp. ***subcanescens*** (DC.).

Osteospermum subcanescens (DC.), incl. var. *virescens* (DC.) Prodr. Regn. Veg. 6: 464 (1837).

Osteospermum moniliferum var. *angustifolia* (DC.), Prodr. Regn. Veg. 6: 460 (1837); Harv. et Sond. Fl. Cap. 3: 436 (1865).

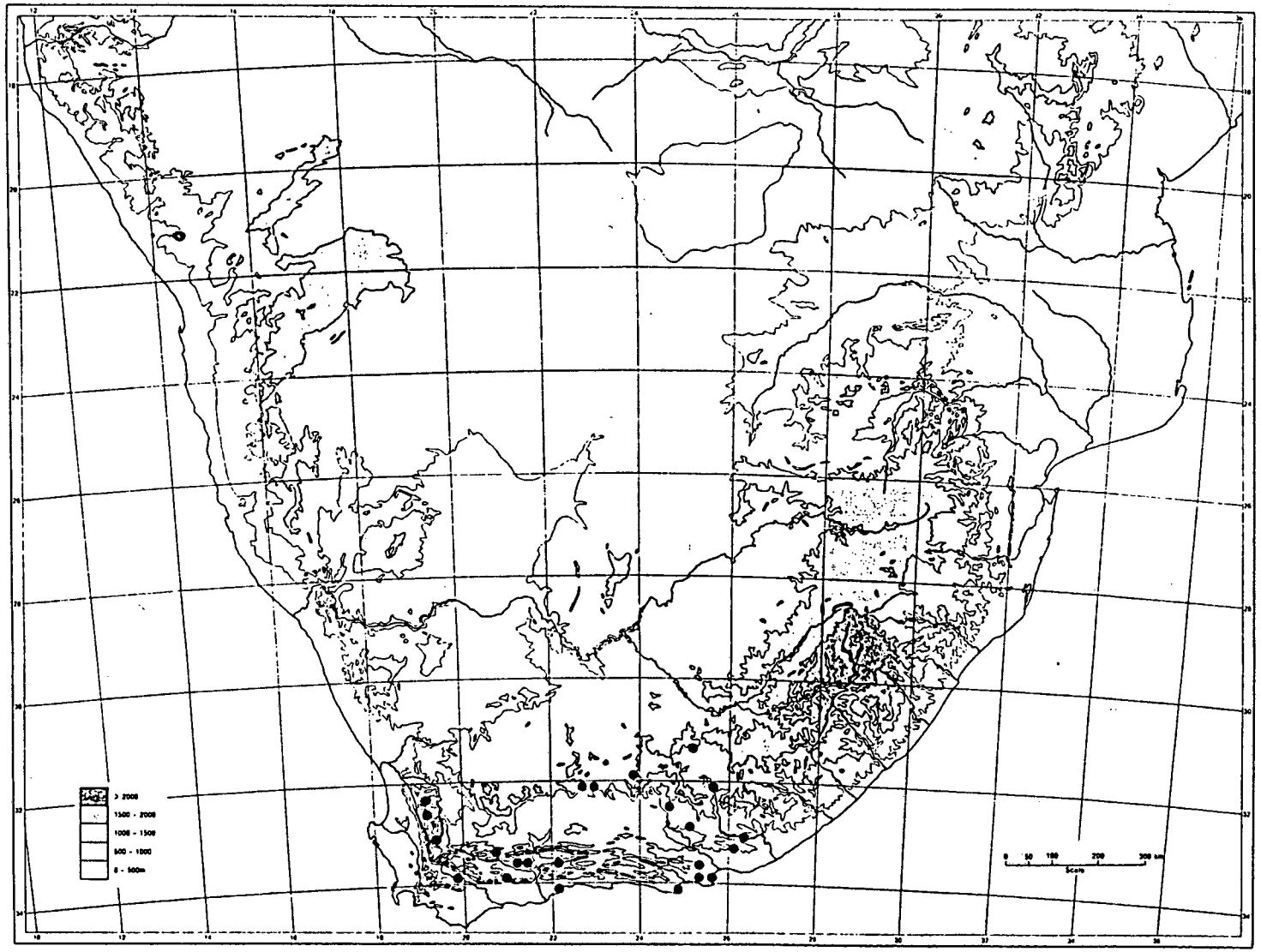
Chrysanthemoides monilifera ssp. *subcanescens* (DC.) T. Norl.,Stud. Calend.: 390 (1943).

Type: Beaufort West, Renosterkop, 750 - 900 m, Drège 692 (G).

Bushes up to 2.5 m high, spreading, compact, young tissue pubescent. *Stems* up to 2 m long, glabrous, tannins absent. *Leaves* narrowly elliptic, 0.5 - 1.0 mm thick, 3 - 10 mm X 10 - 32 mm, glabrous, pubescent, tannins absent, leaf margins minutely spinescent or entire, petiole 2 - 10 mm long. Inner *involucral scales* ovate, outer involucral scales lanceolate, 2.5 - 7.0 mm long. *Drupe* ovoid, 4 - 6 mm long, drupe length/drupe breadth ratio 2.8 - 3.0, drupe ridging present.

Ecology and Distribution: I have not seen the subspecies in their natural habitat, therefore little comment is offered on growth habit. However, plants have been found growing in the Swartkops River bed, Outshoorn, Uitenhage, Middelberg, Mossel Bay, Graaff Reinet and Robertson (Map 13). The ecology of the subspecies is not fully understood because of its wide distribution and variety of vegetation types it occurs in. The subspecies grows in well drained gravels derived from shale or sandstone at Port Elizabeth. Plants occur at altitudes ranging from 50 - 1000 m above sea level.

Selected specimens: Eastern Cape, Uitenhage, Swartkops River, *Ecklon & Zeyher* 39818 (BOL); Western Cape, Cowlauds Poort, Nelspoort, 1907, *Pearson* 2040 (BOL); Eastern Cape, Humansdorp, Mistkraal, 1953, *Compton* 24069 (NGB); Western Cape, Darling, Pampoenvlei, 1944, *Henrier* 3760 (NGB).



Map 13: Distribution of *C. incana* ssp. *subcanescens*.

5.3. Populations Showing Phenotypic Plasticity

***C. monilifera* ssp. *pisifera* var. *pisifera* (form 1) X *C. monilifera* ssp. *rotundata*.** The above taxa morphologically intergrade with one another at the margins of their distribution. Environmental factors limiting the taxa's distribution, are possibly related to soil nutrition, since coastal plants are found growing on sand dunes, unlike *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1) inhabiting inland, fertile soils. A distributional overlap percentage of 12.9 % was calculated (Table 11). Plants were collected 22 km inland on the outskirts of Port Elizabeth (*Fourcade* 391; *Giffen* 62). Leaves are broadly elliptic and leaf margins are scolloped. Pubescence is absent from leaves and stems. Involucral scales are distinctly oval and pubescent.

***C. monilifera* ssp. *rotundata* X *C. monilifera* ssp. *canescens*.** Populations are found in southern and central Natal occurring on the Drakensberg escarpment. Specimens with a similar morphology have been collected in the Umtamvuna Nature Reserve and Pietermaritzburg, World View. Morphological adaptation at higher altitudes is suggested for the subspecies. Intermediate populations resemble *C. monilifera* ssp. *floribunda* (form 1) collected at Rooiels (*Oliver* 5112). Plants have small, broadly obovate leaves clustered at branch terminals (*Abbott* 2518; *Galpin* 21699; *Ward* 7182).

***C. monilifera* ssp. *floribunda* (form 2) X *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1).** Plants have been collected in the Keurbooms River vicinity in close proximity of the coast. Increased humic content of soils, higher rainfall averages per annum and shaded environments could induced morphological change. A distribution overlap of 14.9 % (Table 11) was calculated for the two subspecies. Intermediates have broadly elliptic leaves and involucral scales are ovate and pubescent (*Gillett* 4549; *O'Callaghan* 19; *Rycroft* 2605)

***C. monilifera* ssp. *pisifera* var. *pisifera* (form 2) X *C. monilifera* ssp. *canescens*.** Collected specimens from Roodekop in the Little Karoo are intermediate in morphology. Plants were collected at 1000 m above sea level. The high altitude and low annual rainfall are suggested environmental factors causing morphological adaptation. Plants are pubescent on their stems and abaxial surfaces of their leaves, and leaves are leathery which is characteristic of *C. monilifera* ssp. *pisifera* var. *pisifera* (form 2) (*Levyns* 6089).

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Appendix 1: Characters used in PCA and Cluster Analysis.

Characters used in PCA and cluster analysis 2 (Fig. 2 & 4) are highlighted with an asterisk (*) (Fig. 1 - 4):

Branch terminals and growth habit. Spinescent taxa are either spinescent or have pungent branch terminals. The two characters were listed as separate, their presence scored with a 1 and absence with a 0. Spinescent plants in most cases have a prostrate growth habit. *C. incana* ssp. *incana* var. *microphylla*, forms a dome-shaped shrub, while *C. incana* ssp. *subcanescens* can reach a height of 2.5 m with branches up to 2 m in length. **spinescence present/absent; pungent branch terminals present/absent; prostrate growth form present/absent.**

Pubescence and spinescence. Pubescence was measured by scoring the degrees of thickness, scaled from 1 to 3. The following pubescence and spinescence characters were used: *** pubescence on adaxial leaf surface; * pubescence on abaxial leaf surface; * pubescence on stem; * pubescence on receptacle.**

Drupe size and shape. Previous systematists have made use of drupe size and shape for taxonomic separation of subspecies of *Chrysanthemoides* (Miller 1759; Norlindh 1943). *C. monilifera* ssp. *monilifera* has globose drupes and occurs at higher altitudes close to the coast. *C. monilifera* ssp. *pisifera* has elongated drupes like most other *Chrysanthemoides* taxa (Fig. 14). Herbarium specimens not examined in their fruiting stage, were scored with a 999. Drupes were assessed as follows: **drupe length/drupe breadth ratio; * drupe ridging.**

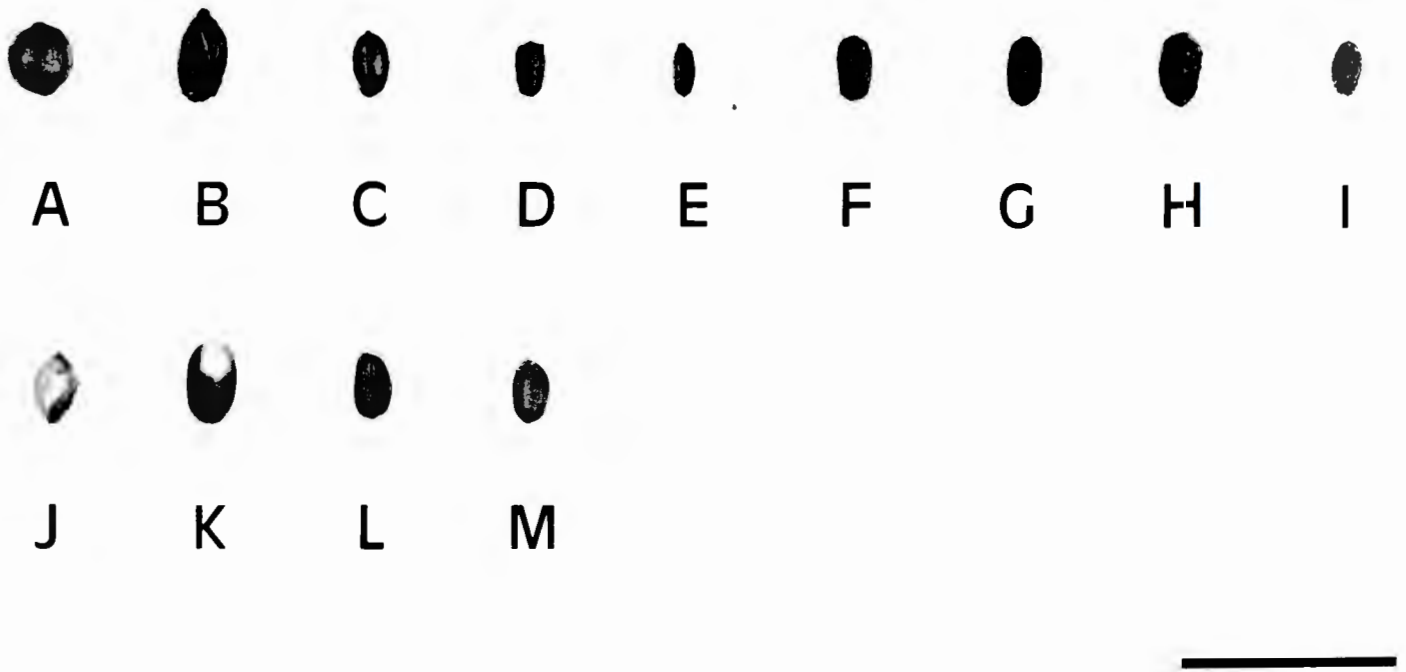

Figure 14: *Chrysanthemoides* drupe shape. A - *C.mon.mon.*; B - *C.mon.flo.*F2; C - *C.mon.flo.*F1; D - *C.mon.pis.*F1; E - *C. mon.pis.*F2; F - *C.mon.can.*; G - *C.mon.sep.*; H - *C.mon.rot.*; I - *C.mon.pis.ang.*; J - *C.inc.inc.*; K - *C.inc.mic.*; L - *C.inc.ran.*; M - *C.inc.sub.*; scale bar = 2 cm; (see Table 1b for abbreviations of taxon names).

Involucral scales. Involucral scales have been widely used in taxonomy, particularly at the species level. The degree of overlap, thickness and ribbing of phyllaries were used for characterizing *Gerbera* and *Senecio* species (Compositae) (Hansen 1985; Belcher 1993). Phyllaries are absent from *Chrysanthemoides* involucral scale surfaces. Involucral scale shape does not represent sufficiently large morphological gaps for the separation of taxa of *Chrysanthemoides*. However, *C. monilifera* ssp. *pisifera* var. *angustifola*, *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1) and *C. monilifera* ssp. *floribunda* (form 1 & 2) involucral scale shape in conjunction with their distribution, are diagnostic.

No variation in row number and imbrication of the involucral scales was recorded. Involucral scale shape was described as linear, lanceolate or ovate. Ratios of the larger inner involucral scale were calculated against length of outer involucral scales. Measurements included: **shape of outer involucral scale; shape of inner involucral scale; inner involucral scale length/outer involucral scale length ratio.**

Bracteoles and phyllaries. Calycular bracteoles and phyllaries have been used for separating lautusoid *Senecio* of Australia from *S. lautus* sensu stricto of New Zealand (Belcher 1993). Phyllaries are absent from the surfaces of involucral scales, and bracteoles although present, are without morphological variation in *Chrysanthemoides*.

Leaf characters. Leaf shape, pubescence, colour and density were used to describe species of *Gerbera* (Compositae) (Hansen 1985). The variation occurs in leaf shape and size in *Chrysanthemoides* is substantial (Figs. 15 - 18). Most leaf measurements are presented in ratio form to prevent bias in PCA and cluster analysis due to environmental adaptation.
To quantify the size of leaf margin serrations, the width of the leaf at the widest tooth serration and its widest sinus was measured. The number of leaf margin serrations was recorded for

one side of the leaf. Margins were toothed (dentate), entire, spinescent, or scolloped at regular intervals. Leaf and petiole size were measured. Leaf shape was described as narrowly elliptic, broadly elliptic, ovate, narrowly obovate, obovate or broadly obovate. Sections of leaves were prepared so the thickness of leaves and their cuticles could be measured. Leaf characters are: *** leaf shape; * leaf length/leaf breadth ratio; * leaf length/petiole length ratio; * leaf width/leaf width at inner sinus ratio; leaf margin type; * number of teeth on one side of leaf margin; * petiole length; leaf thickness/cuticle thickness ratio; leaf length/leaf thickness ratio; leaf colour.**

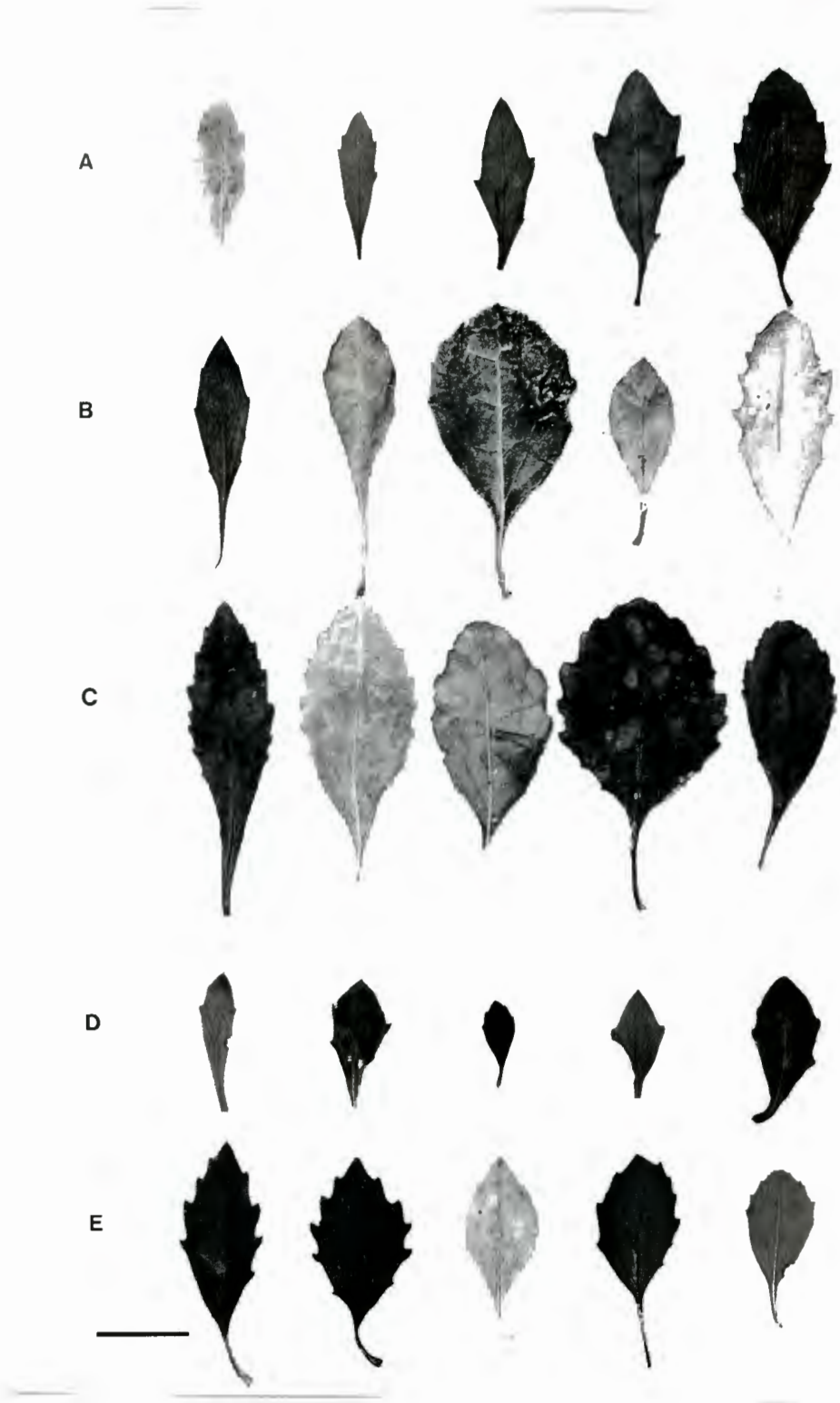

Figure 15: Leaf variation for taxa of *C. monilifera*. A - *C.mon.mon.*; B - *C.mon.flo*.F1; C - *C.mon.flo*.F2; D - *C.mon.pis*.F1; E - *C.mon.pis*.F2; scale bar = 2 cm; (see Table 1b for abbreviations of taxon names).

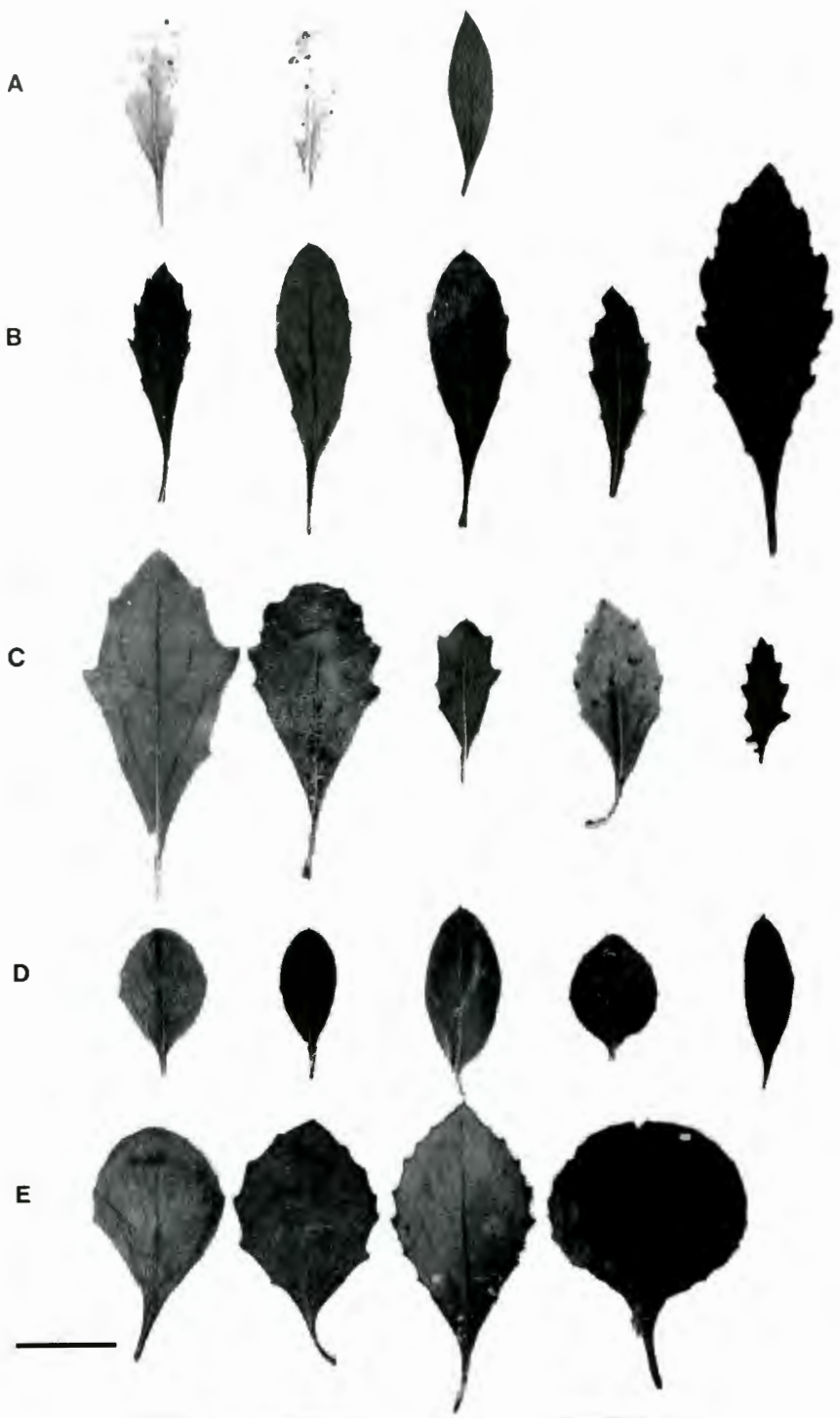

Figure 16: Leaf variation for taxa of *C. monilifera*. A - *C.mon.pis.bor.*; B - *C.mon.pis.ang.*; C - *C.mon.can.*F2; D - *C.mon.sep.*; E - *C.mon.rot.*; scale bar = 2 cm; (see Table 1b for abbreviations of taxon names).

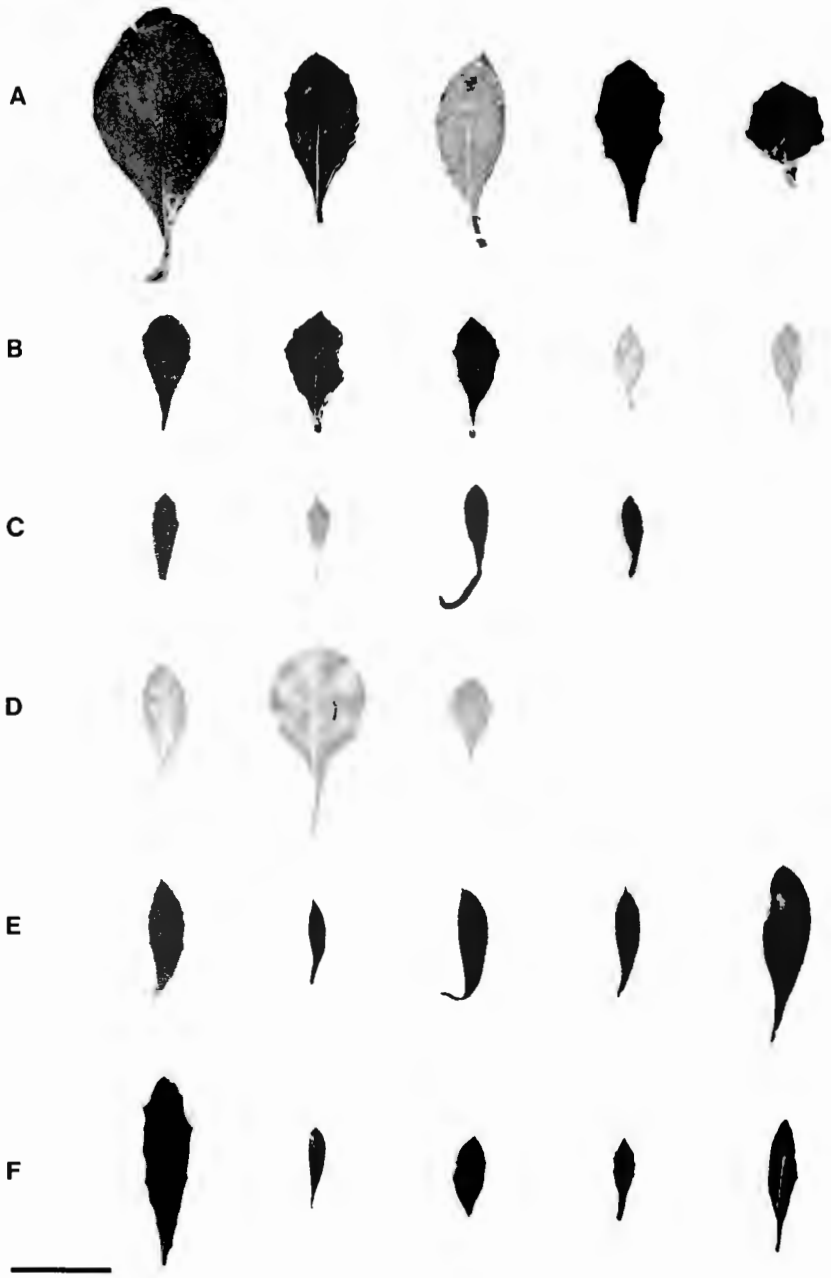

Figure 17: Leaf variation for taxa of *C. incana*. A - *C.inc.inc.* B - *C.inc.mic.*; C - *C.inc.ran.*; D - *C.inc.hir.*; E - *C.inc.gra.*; F - *C.inc.sub.*; scale bar = 2 cm; (see Table 1b for abbreviations of taxon names).

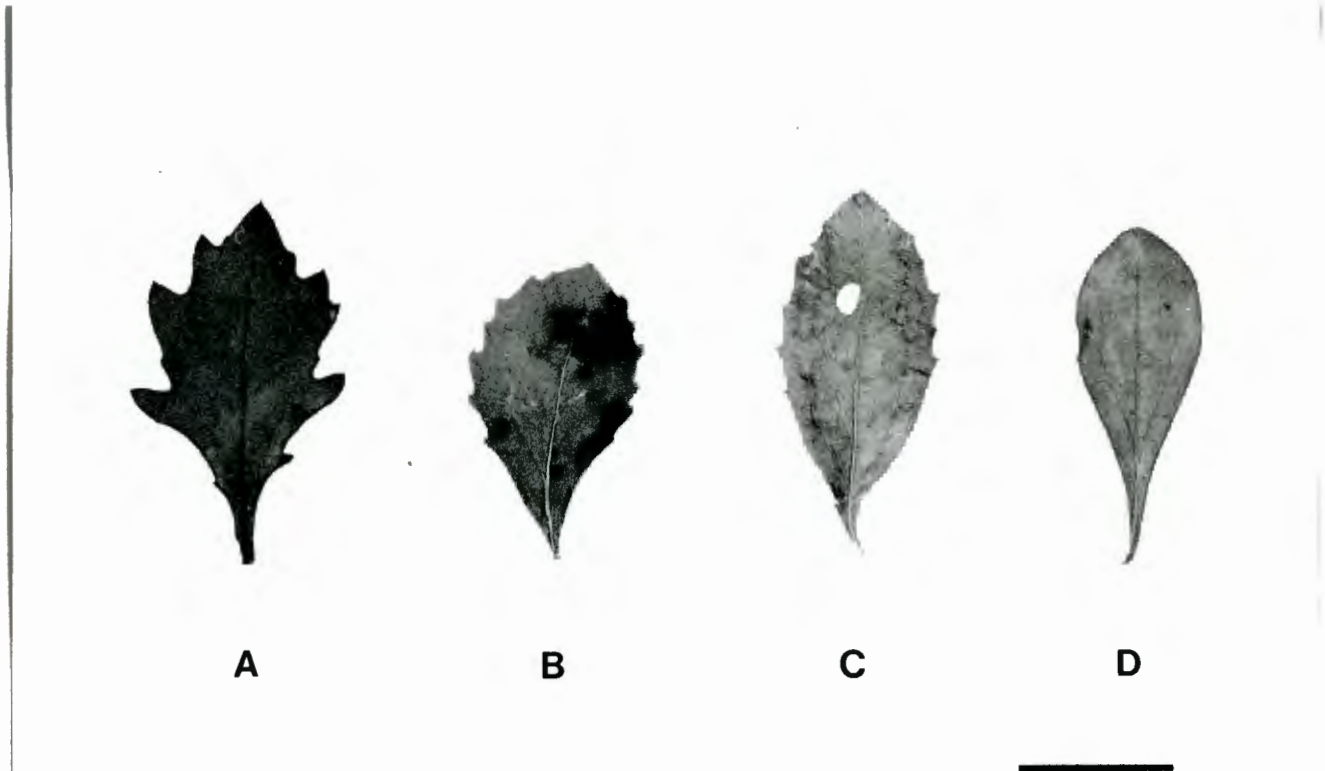

Figure 18: *Chrysanthemoides* leaf margin types. A - toothed/dentate (*C.mon.pis*.F2); B - scolloped (*C.mon.flo*.F1); C - spinescent (*C.mon.sep*.); D - entire (*C.mon.flo*.F1); scale bar = 2 cm; (see Table 1b for abbreviations of taxon names).

Floral features.

Marginal and disc florets. Marginal floret and anther size have been made use of for the classifications of new species of *Brachysome* (Compositae) (Short & Watanabe 1993). Calculations are presented in ratio form to eliminate size differences that might result from environmental modification. Slides of floral material were prepared and mounted in a 50 % aqueous solution of fuschin - glycerol. The following marginal and disc floret measurements were recorded: **number of marginal florets per inflorescence; disc and marginal floret pubescence; * marginal floret length/marginal floret width ratio; marginal floret length/capitulum diameter ratio; marginal floret length/ovary length ratio; disc style length/disc style petal length ratio; * number of inflorescences per branch terminal.**

Anatomy. Anatomy was of little use in PCA (Figs. 3 & 4) and cluster analysis (Figs. 1 & 2), however, tannin distribution in the stems and leaves show variation. *C. monilifera* ssp. *monilifera*, *C. monilifera* ssp. *floribunda* (form 1), *C. monilifera* ssp. *floribunda* (form 2), *C. monilifera* ssp. *pisifera* var. *angustifolia* stem and leaf material are tanniferous. *C. monilifera* ssp. *canescens*, *C. monilifera* ssp. *pisifera* var. *borealis*, *C. monilifera* ssp. *septentrionalis* either have tanniferous stems and leaves, and in certain cases tannins may be absent in both. Members of *C. incana* are without tannins in stem and leaf tissue. Plants are spinescent serving as an anti-herbivory defense mechanism.

Lignification (secondary thickening) and cell anatomy in stems and leaves of taxa were examined. Intracellular crystalline bodies were found in all taxa. Leaf thickness, and diameters of the stems, secondary xylem and piths were measured. The following measurements were taken: **stem diameter/pith diameter ratio; stem**

diameter/primary xylem diameter ratio; leaf tissue tanniferous; stem tissue tanniferous.

Cytology. Norlindh (1963) found that chromosome number in *Chrysanthemoides, Dimorphotheca, Castalis* and *Gibbaria* are the same (2n = 20) which has been found for about half of the species in the tribe. Chromosome number does not supply morphological gaps when classifying genera and species in the Calenduleae.

Table A: Chromosome numbers for some of the closely related genera of *Chrysanthemoides* (Norlindh 1963). Question marks indicate estimates not published yet.

Taxon	Origin	Chromosome Number (n)
Dimorphotheca montana var. *hortensis*	Worcester	9;10 (Nordenstam 1994)
Castalis nudicaulis s. st.	Stellenbosch	10 (T. Norl. 1963)
Osteospermum	-	8;9;12;? (T. Norl. 1963)
Chrysanthemoides	SW Cape	10 (T. Norl. 1963)
Gibbaria	Cape Peninsula	10 (T. Norl. 1963)
Calendula	-	7;8;9;11;15;? (T. Norl. 1977)

Appendix 2: Characters used in the Ecological PCA.

Altitude. Altitude data recordings were analysed so that the taxons entire range of altitude was taken into account. Averages were calculated using the following formula:

$$\text{mean altitude} = x + \frac{(y - x)}{2}$$

where x is the minimum altitude and y the maximum altitude. Characters included: **highest altitude; lowest altitude; average altitude.**

Rainfall. Rainfall was analyzed using the same equation to calculate averages. Distributions of taxa were classified into winter, summer, or winter and summer rainfall areas: **highest rainfall; lowest rainfall; average rainfall; season of rainfall.**

Appendix 3: Characters used in the Phylogenetic Analysis.

Many characters were evaluated but excluded as uninformative, either being autapomorphic for the entire genus or too variable within taxa. To demonstrate monophyly, characters that showed continuous variation gaps were included into the data matrix (Table 13).

1. *Drupe length/drupe breadth ratio*. *Chrysanthemoides* is the only genus of the tribe Calenduleae that produces drupes (Fig 14). Other genera produce achenes or cypsela that may or may not be winged. Marginal florets are fertile while disc florets are sterile and not producing fruit. Drupe ratios calculated for taxa were categorized into three groups: **drupe length/drupe breadth ratio 1.40 (0); drupe length/drupe breadth ratio 1.65 - 2.33 (1); drupe length/drupe breadth ratio 2.75 - 3.0 (2); drupe length/drupe breadth ratio 1 - 1.2 (3).**

2. *Pubescence on adaxial leaf surface*. Pubescence was found on both adaxial and abaxial leaf surfaces, especially for *C. incana* ssp. *incana*. Pubescence on both leaf surfaces was evaluated as one characters since pubescence distribution on abaxial and adaxial surfaces of the leaf are the same. Absence of pubescence appears to be the plesiomorphic state, since stems and receptacles of the outgroup *Osteospermum* were glabrous: **adaxial leaf surface glabrous (0); adaxial leaf surface half pubescent (1); adaxial leaf surface pubescent (2).**

3. *Pubescence on stem material*. Stem pubescence is in most cases the same as the pubescence observed on leaves: **stem surface glabrous (0); stem surface half pubescent (1); stem surface pubescent (2).**

4. *Pubescence on inflorescences*. *C. monilifera* ssp. *floribunda* forms 1 and 2 have a loose pubescence covering the surface of

their receptacles. Receptacles of *C. incana* ssp. *incana* (*C. incana* ssp. *subcanescens* has naked receptacles) are densely covered with pubescence: **receptacle surface glabrous (0); receptacle surface half pubescent (1); receptacle surface pubescent (2).**

5. *Leaf shape*. All inland taxa have elliptic or slightly obovate leaves in contrast to the slightly obovate leaves (Figs. 15 - 17) of coastal taxa (*C. monilifera* ssp. *floribunda* (form 1); *C. monilifera* ssp. *rotundata*; *C. incana* ssp. *incana* (excluding *C. incana* ssp. *incana* var. *gracilis*)): **leaf shape elliptic (0); leaf shape obovate (1).**

6. *Leaf margin*. Leaf margins (Fig. 18) are often characteristic of various taxa, and the variation has been classified as follows: **leaf margin dentate (0); leaf margin scolloped (1); leaf margin entire (2).**

7. *Spinescence*. The transformation of branch apices into spines usually takes place when branches are poorly developed. Branches are pungent and sometimes leafless. When the peduncles lignify after inflorescences have fallen, they form a spine: **plants spinescent (0); plants non-spinescent (1).**

8. *Leaf width*. Leaves may either be broadly or narrowly obovate. Coastal taxa have broad, obovate leaves (*C. monilifera* ssp. *floribunda* (form 1); *C. monilifera* ssp. *rotundata*; *C. incana* ssp. *incana* (excluding *C. incana* ssp. *incana* var. *gracilis*)). Leaves with entire and scolloped leaf margins are usually narrow (*C. monilifera* ssp. *septentrionalis*; *C. incana* ssp. *incana* var. *gracilis*; *C. monilifera* ssp. *pisifera* var. *angustifolia*; *C. monilifera* ssp. *floribunda* (form 2)): **leaves narrow (0); leaves broad (1).**

9. *Habitat*. Habitat data was deactivated for phylogenetic analysis. Only morphological data was incorporated into the cladistic analysis.

10. *Leaf texture*. Taxa were classified according to their leaf texture. Cases do occur where a taxon has both leathery and non-leathery leaves. Older leaves of *C. monilifera* ssp. *floribunda* form 1 are usually leathery while younger leaves are partially leathery. In these cases mature leaves were scored: **leaves leathery (0); leaves not leathery (1).**

11. *Habit*. Habit is often variable. *C. incana* has a prostrate growth form, except *C. incana* ssp. *incana* var. *microphylla* which forms a dome-shaped shrub (up to 2 m in height) and *C. monilifera* ssp. *subcanescens* which forms a large spreading shrub (up to 2.5 m in height), but still has prostrate branches. *C. monilifera* forms a tree or shrub: **shrub (0); prostrate growth (1).**

12. *Tannins in stem material*. Tannin content of *C. monilifera* ssp. *monilifera*, *C. monilifera* ssp. *pisifera* and *C. incana* has been previously determined (Moolman & Stock 1991). In all the species, tannin content was low (0.5% of d.w). Indications are that *C. incana* has a lower content than other taxa assessed. However, plants are spinescent which may substitute the absence of tannins, acting as an anti-herbivory mechanism. Information on epidermis types, seed oils, low molecular weight constituents and other compounds of *Chrysanthemoides* is outlined in 'The Biology and Chemistry of the Compositae' (Heywood *et al*. 1977). : **tannins absent in stem material (0); tanniferous stem material (1).**

13. *Tannins in leaf material:* **tannins absent in leaf material (0); tanniferous leaf material (1).**

14. *Leaf emargination*. The measurement describes the size of leaf margin indentations. Leaf width was measured at its widest

outdent and its adjacent inner sinus. Measurements were presented in ratio form: **widest width of leaf/width of leaf at widest sinus ratio 1 - 1.09 (0); widest width of leaf/width of leaf at widest sinus ratio 1.13 - 1.33 (1).**

15. *Leaf length/petiole length ratio.* *Osteospermum* sect. *Homocarpa* leaves were sessile and coded with an inapplicable question mark (?) in the data matrix. Petiole length of *Chrysanthemoides* were presented in ratio form with leaf length: **leaf length/petiole length ratio > 4 (0); leaf length/petiole length ratio 2 - 4 (1).**

16. *Drupe ridging.* Fine ridging on the surface of the drupes may occur. Drupe ridging prominence varies for different subspecies, with the character state absent on *C. incana* drupes while *C. monilifera* ssp. *septentrionalis*, *C. monilifera* ssp. *canescens* and *C. monilifera* ssp. *rotundata* drupes are conspicously ridged. Character recorded are: **drupe ridging absent (0); drupe ridging present (1).**

17. *Involucral inner and outer scale ratio.* Norlindh (1943) used involucral scale shape to classify *C. monilifera* and *C. incana*. *C. monilifera* ssp. *pisifera* var. *pisifera* (form 1), *C. monilifera* ssp. *floribunda* (form 2) and *C. monilifera* ssp. *pisifera* var. *angustifolia* have ovate inner and outer involucral scales. *C. monilifera* ssp. *pisifera* var. *pisifera* (form 2) and *C. monilifera* ssp. *pisifera* var. *borealis* have lanceolate inner and outer scales. Involucral scale size and shape was quantified as ratios: **1.25 - 1.3 (0); 1.4 - 1.5 (1); 1.6 - 1.7 (2); 2 (3).**

18. *Fruit type.* The Calenduleae is the most complex tribe in the Compositae with regard to fruit type. Fruits develop from disc achenes which are bilateral, and ray achenes which are trilateral or terete (Norlindh 1977). *Chrysanthemoides* differs in that it is

the only known genus that develops a drupaceous fruit in the family: **achenes present (0); drupes present (1).**

19. *Marginal floret length/capitulum diameter ratio.* Taxa found growing on forest margins, *C. monilifera* ssp. *floribunda* (form 2), and *C. monilifera* ssp. *pisifera* var. *pisifera* have long marginal florets. *C. incana* have short marginal florets which roll abaxially with desiccation: **1.0 - 1.2 (0); 1.4 - 2.3 (1).**

20. *Leaves sessile or petiolate.* The outgroup species, *O. ciliatum* and *O. grandidentatum* both have sessile leaves, while all *Chrysanthemoides* taxa have petiolate leaves: **sessile leaves (0); petiolate leaves (1).**

21. *Achene ridging.* This character specifically applies to the two *Osteospermum* sect. *Homocarpa* outgroups: **Achene ridging absent (0); achene ridging present (1).**

22. *Stellate hairs on leaves.* Stellate hairs were found on adaxial and abaxial leaf surfaces of *Osteospermum* sect. *Homocarpa* taxa. Stellate hairs were not found on *Chrysanthemoides* leaves: **stellate hairs absent from leaf surfaces (0); stellate hairs present on leaf surfaces (1).**

23. *Pungent branch terminals.* Pungent branch terminals are associated with *C. incana.* This character strengthens the split between spinescent (*C. incana*) and non-spinescent (*C. monilifera*) taxa: **branch terminals not pungent (0); branch terminals pungent (1).**

Appendix 4: Enzymes used and Formulations for Buffers, Stock Solutions and Stains.

4.1. Enzymes

The following enzymes were tested electrophoretically. Staining patterns were found for enzymes highlighted with an asterisk (*)

- aconitase (ACON)
- alcohol dehydrogenase (ADH)*
- acid phosphatase (ACP) *
- alanine aminopeptidase (AAP)
- aldolase (ALD) *
- catalase (CAT)
- alpha-esterase (alpha-EST) *
- beta-esterase (beta-EST) *
- glutamate dehydrogenase (GDH) *
- glutamate-oxaloacetate transaminase (GOT) *
- glucose-6-phosphate dehydrogenase (G6PD) *
- leucine aminopeptidase (LAP) *
- malic dehydrogenase (MDH) *
- 6-phosphogluconate dehydrogenase (6PGD)
- phosphoglucomutase (PGM) *
- phosphoglucose isomerase (PGI) *
- superoxide dismutase (SOD) *
- shikimate dehydrogenase (SKDH)

Stains for the above enzymes were formulated according to the laboratory rules described by Conkle *et al.* (1982). Chemical components were purchased from Sigma Chemical Co., P.O. Box 14508, St Louis, Mo. 63178.

4.2. Grinding Buffers

4.2.1. Tris-HCl Buffer

The enzymes ACP (acid phosphatase), ADH (alcohol dehydrogenase), alpha-EST (alpha esterase), GOT (glutamateoxaloacetate transaminase), G6PD (glucose-6-phosphate dehydrogenase) and PGM (phosphoglucomutase) were assayed using a tris-HCl buffer system. Young leaf material from plants several weeks of age were ground in a buffer consisting of 0.1 M Tris-HCl (pH = 7.5), 14 mM 2 - mercaptoethanol, 1.0 mM EDTA (tetrasodium salt), 10 mM KCl and 5 - 10 mg polyvinylpolypyrrolidone per 0.5 ml buffer (Gottlieb 1981).

4.2.2. Tris-Malate Buffer

The enzymes ALD (aldolase), beta-EST (beta esterase), GDH (glutamate dehydrogenase), PGI (phosphoglucose isomerase) and SOD (superoxide dismutase) were assayed using an tris-malate grinding buffer. The buffer consisted of 0.1 M tris-HCl, 0.2 M sodium tetraborate, 0.2 M sodium metabisulphite, 0.25 M sodium ascorbate, 0.026 M diethyldithio carbamate, 0.28 M mercaptoethanol and 1 g polypyriolidone. This was made up to 25 ml distilled water and adjusted to pH 7.5 with 1 M HCl (modified from Werth 1985).

4.3. Gel and Tray Buffer Formulations (Conkle *et al.* 1982)

System	Gel Buffer	Tray Buffer
A	**Tris citrate (pH = 8.3)**	**Lithium Borate (pH = 8.3)**
Formulation	Trizma base.....12.4g Citric acid.....2.72g Distilled water.....2l	Lithium hydroxide....2.4g Boric acid.....28.72g Distilled water.....2l
Procedure	To use, add 50ml of the lithium borate tray buffer to 400ml of the tris citrate buffer to make the required 450ml	
B	**Tris citrate (pH = 8.8)**	**Sodium Borate (pH = 8.0)**
Formulation	Trizma base.....24.2g Distilled water.....2l	Sodium hydroxide.....4g Boric acid.....37.1g Distilled water.....2l
	Titrate to pH 8.8 with 0.2M citric acid solution	Titrate to pH 8.0 with 4N NaOH
C	**Tris citrate (pH = 6.2)**	**Same as gel buffer**
Formulation	Trizma base.....32.4g Citric acid.....21.78g Distilled water...600ml	
	Titrate to pH 6.2 with 4N NaOH	
Procedure	Mix 3.2ml of buffer with 146.8ml of distilled water	Mix 50ml of buffer with 150ml distilled water
D	**Morpholine citrate (pH 6.1)**	**Same as gel buffer**
Formulation	Citric acid.....3.08g Distilled water....500ml Dissolve citric acid and titrate to pH 6.1 with N-(3-aminopropyl) morpholine (4ml). Refrigerate.	Same as gel buffer
Procedure	Mix 7.5ml of buffer with 142.5ml distilled water	

4.4. Stock Solutions for Staining Components (Conkle *et al.* 1982)

Abbreviation	Name	Standard concentration
G6PDH	Glucose-6-phosphate dehydrogenase	5 units/ml buffer
NAD	beta-Nicotinamide adenine dinucleotide	10 mg/ml water
NADP	beta-NAD phosphate	10 mg/ml water
NBT	Nitro blue tetrazolium	10 mg/ml water
PMS	Phenazine methosulfate	5 mg/ml water

4.5. Staining Buffer Formulations (Conkle *et al.* 1982)

Buffer	pH	Formulation
ACP acetate buffer	4.0	Sodium acetate.....2.42g Acetic acid, glacial...4.7g 1.0M magnesium chloride .....5.0ml Distilled water.....1.0l
1.0M tris Hydrochloride	8.0	Trizma base.....16.0g Trizma hydrochloride..61.4g Distilled water.....1.0l
Amino peptidase	4.5	Trizma base.....12.1g Maleic anhydride.....9.8g Sodium hydroxide.....1.6l Distilled water.....1.0l
Catalase buffer	6.5	Sodium phosphate monobasic.....18.5g Sodium phosphate dibasic.....17.9g Distilled water.....1.0l
Esterase buffer	6.4	Sodium phosphate monobasic.....13.9g Sodium phosphate dibasic.....5.3g Distilled water.....1.0l
0.2M Phosphate buffer	7.5	Sodium phosphate monobasic.....3.84g Sodium phosphate dibasic.....23.86g Distilled water.....1l
1.0M Tris hydrochloride	7.0	Trizma base.....16.0g Trizma HCl..... 137.4g Distilled water.....1l

4.6. Stain Recipes (Conkle *et al.* 1982)

Enzyme	Gel buffer	Stain buffer	Stain components	Procedure
ACON	C;D	75ml 0.2 M tris HCl pH 8.0	Cis-aconitic acid.....150mg NADP.....1ml NBT.....1ml 1% MgCl....1ml PMS.....0.5ml Isocitrate dehydrogenase... .....20 units	Add components to warm stain buffer and incubate gels at 37 °C in the dark.
ACP	B	80ml ACP acetate buffer	alpha-napthyl acid phosphate .....100mg Fast Garnet GBC salt....100mg	Develop at room temperature. Incubate cathodal slice of gel.
ADH	A	75ml 0.05M tris HCl pH 8.0	NAD.....1ml NBT.....1ml PMS.....0.5ml 95% ETOH..1ml	Add components to warm buffer and incubate gels in the dark.
AAP	A	75ml amino-peptidase buffer	L-alanine beta-naphthylamide .....30mg dissolved in dimethyl-sulfoxide...2ml Fast Black K salt.....20mg	Add components to warm buffer and incubate gels in the dark.
ALD	C;D	75ml 0.05M tris HCl pH 8.0	D-fructose-1,6-diphosphate..... .....250mg Arsenic acid .....75mg NAD .....1ml NBT.....1ml PMS.....0.5ml Glyceraldehyde-3-phosphate dehydrogenase... .....300 units	Add components to warm buffer and incubate gels.

CAT	B	100ml catalase buffer	2% potassium iodide solution .....100ml 0.03% H_2O_2 solution..100ml	Refrigerate gels in catalase buffer for 30 min. Drain off buffer and soak in KI for 2 min. Drain off KI and wash twice in tap water. Add H_2O_2.
alpha-EST	A	75ml esterase buffer	Fast blue RR salt.....80mg 1% alpha naphthyl acetate solution....1ml	Add components to warm buffer. Add naphthyl solution late in mixing. Incubate gels.
beta-EST	A	75ml esterase buffer	Fast Garnet GBC salt.....100mg 1% beta-naphthyl acetate solution....1ml	Add components to warm buffer. Add naphthyl acetate solution just before adding gels.
GDH; SOD	B	75ml 0.1M tris HCl pH 8.0	L-glutamic acid.....2g NAD.....1ml NBT.....1ml PMS.....0.5ml	Add components to warm buffer.
GOT	B	75ml 0.2M phosphate buffer pH 7.5	0.5% pyridoxal 5-phosphate solution..0.8ml 3% bovine albumine..1.6ml 0.2 M L-asparatic acid (adjusted to pH 7.5 with 2N KOH) .....6.8 ml alpha-ketoglutarate... .....2ml Fast blue BB salt.....120mg dissolved in distilled water 8ml	Combine first four components with buffer. When ready for staining add the Fast Blue BB solution to the component solution. Add gels and develop at room temperature. Include a cathodal slice of gel.

G6PD	B	75ml 0.05M tris HCl pH 7.0	D-glucose-6-phosphate..20mg 3.0% bovine albumine solution...1ml NADP.....1ml NBT.....1ml 1% $MgCL_2$...1ml PMS.....0.5ml	Add components to warm buffer and incubate gels in the dark.
LAP	A;D	75ml Amino-peptidase buffer	0.4% L-leucine beta-napthylamide solution...5ml Black K salt 20mg	Add components to warm buffer and incubate gels in the dark.
MDH	C;D	75ml 0.05M tris HCl pH 8.0	Malic acid solution....5ml (134.1g DL-malic acid, 80g NaOH, 1.0l H_2O at pH 7 with 18ml of 4N NaOH) NAD.....1ml NBT.....1ml PMS.....0.5ml	Add components to warm buffer and incubate gel in the dark.
6PGD	C;D	75 ml 0.05M tris HCl pH 8.0	6-phospho-gluconic acid .....20mg NADP.....1ml NBT.....1ml PMS.....0.5ml	Add components to warm stain buffer, add gels and incubate in the dark.
PGI	A	75ml 0.05M tris HCl pH 8.0	D-fructose-6-phosphate...25mg 1% MgCl solution .....1ml NADP.....1ml NBT.....1ml PMS.....0.5ml G6PDH...20 units	Add all components to warm buffer. Add gels and incubate in the dark.
PGM	A	75ml 0.05M tris HCl pH 8.0	alpha-D-glucose 1,6-diphosphate solution (10mg, 100ml distilled water).....1ml alpha-D-glucose 1-phosphate 140mg	Add all components to warm buffer. Add gels and incubate in the dark.

SKDH	C;D	75ml 0.05M tris HCl pH 8.0	Shikimic acid ....75 to 50mg NADP.....1ml NBT.....1ml PMS.....0.5ml	Add all components to warm buffer and incubate in the dark.

Appendix 5: List of Exsiccate Studied.

(1 - *C. monilifera* ssp. *monilifera*; 2 - *C. monilifera* ssp. *floribunda* forms 1 and 2; 3 - *C. monilifera* ssp. *pisifera* forms 1 and 2; 4 - *C. monilifera* ssp. *pisifera* var. *borealis*; 5 - *C. monilifera* ssp. *pisifera* var. *angustifolia*; 6 - *C. monilifera* ssp. *canescens*; 7 - *C. monilifera* ssp. *septentrionalis*; 8 - *C. monilifera* ssp. *rotundata*; 9 - *C. incana* ssp. *incana* var. *incana*; 10 - *C. incana* ssp. *incana* var. *microphylla*; 11 - *C. incana* ssp. *incana* var. *rangei*; 12 - *C. incana* ssp. *incana* var. *hirsuta*; 13 - *C. incana* ssp. *incana* var. *gracilis*; 14 - *C. incana* ssp. *subcanescens*.

Abbott 1662 (8); 2518 (8); 3751 (8); 3803 (7). Acocks 11516 (6). Admiraal & Dryfhout 2786 (8). Aitken & Gale 8 (8). Allardice 1692 (3). Andreae 991 (3). Archibald 3186 (14); 4219 (8). Atherstone 7653 (3). Atkin 9 (8). Atkinson & Meidner 9427 (8); 9427 (8).

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